SCIENTIFIC OPINION

Guidance on the risk assessment of genetically modified microorganisms and their products intended for food and feed use[1]

EFSA Panel on Genetically Modified Organisms (GMO)[2, 3, 4]

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ABSTRACT

Genetically modified microorganisms (GMMs) are involved in the production of a variety of food and feed. The marketing of these products within the European Union falls under different legislative instruments, which establish the requirement for a risk assessment for the authorisation of the product. The present guidance describes the principles to be followed when conducting such a risk assessment, as well as the scientific information required in applications for authorisation to be evaluated by the Scientific Panel on Genetically Modified Organisms of the European Food Safety Authority (EFSA GMO Panel). Products form four categories, depending on their nature and the level of scientific information required for their evaluation by the EFSA GMO Panel. The guidance details the data to be provided for the assessment of products of each category, providing reference to other guidance that is also applicable. This document draws on the experience gained by the EFSA GMO Panel in assessing applications for marketing food and feed involving GMMs and takes into account the input received from different stakeholders, and updates the "Guidance Document for the risk assessment of genetically modified microorganisms and their derived products intended for food and feed", adopted by the EFSA GMO Panel in 2006.

KEY WORDS

GMOs, GM microorganisms, GM food and feed, guidance, Regulation (EC) No 1829/2003, Directive 2001/18/EC, food safety, feed safety, environment.

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[1] On request from EFSA, Question No EFSA-Q-2009-00521, adopted on 25 May 2011.

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[4] Detlef Bartsch was member of the Updated GMM GD Working Group until 28 March 2011.

Suggested citation: EFSA Panel on Genetically Modified Organisms (GMO); Scientific Opinion on Guidance on the risk assessment of genetically modified microorganisms and their products intended for food and feed use. EFSA Journal 2011;9(6):2193 [54 pp.] doi:10.2903/j.efsa.2011.2193. Available online: http://www.efsa.europa.eu/efsajournal.htm

SUMMARY

The European Food Safety Authority (EFSA) asked the EFSA Panel on Genetically Modified Organisms (EFSA GMO Panel) to establish a 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 encompasses providing updated guidance for the preparation and presentation of applications involving genetically modified microorganisms (GMMs).

The Working Group: i) reviewed the existing Guidance Document in the light of experience gained, technological progress and scientific developments, ii) considered input received from the Member States and applicants on the existing Guidance Document, and iii) completed a public consultation of the draft Guidance.

Guidance for the preparation of applications is given throughout the different chapters of the document. Chapter I clarifies the scope of the document. This guidance covers GMMs and their products falling under Regulation (EC) No 1829/2003 on GM food and feed, as well as those falling under Regulations 1332/2008 (on food enzymes), 1333/2008 (on food additives), 1334/2008 (on food flavourings), and 1831/2003 (on feed additives) when GMMs are involved. Chapter II describes the strategy, the steps to be taken, and the issues to be considered when carrying out a comprehensive risk characterisation. GMMs and their products are divided in four categories, depending on their nature and the level of scientific information required for their risk assessment. Depending on the category to which a product is allocated, its use, and the legislations applicable to the product, it is specified which Sections of this guidance document must be followed, and what other guidelines and guidance are also applicable. Chapter III describes the scientific information that should be provided in applications, and which is required for the risk assessment by the EFSA GMO Panel. This should include a comprehensive characterisation of the GMM and cover the recipient/parental organism, the donor(s) of the genetic material, the genetic modification, and the final GMM and its phenotype. 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.

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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[5] 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 food enzymes, additives, vitamins and flavourings produced by the GMMs.

To date, a number of applications under Regulation (EC) No 1829/2003, as well as under other Regulations, involving GMMs, have been assessed by the EFSA GMO Panel according to the earlier GMM Guidance Document. During this period, the EFSA GMO Panel has gained significant experience, enabling it to identify areas of the Document that needed to be clarified and issues that should be covered more in depth. In addition, the EFSA GMO Panel has received and acknowledged input from different stakeholders, including applicants and Member States, on possible refinements of the 2006 Guidance Document. The EFSA GMO Panel has committed itself to review this guidance regularly in the light of experience gained, technological progress and scientific developments.

A draft updated Guidance was published on the EFSA website from 29th November 2010 until 31st January 2011 for public consultation. The outcome of the public consultation, including a table of all comments received, is published on the EFSA website http://www.efsa.europa.eu (EFSA, 2011).

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 Guidance. In addition, the EFSA GMO Panel follows this Guidance when evaluating the assessments 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.

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 GMMs and their derived products intended for food and feed use. In particular, the EFSA GMO Panel proposed:

1. to update its existing Guidance Document for the risk assessment of GMMs and their derived products intended for food and feed use (EFSA, 2006) with precise guidelines on the risk assessment of GMMs 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.

[5] Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. OJ L 268, 18.10.2003, p. 1-23.

I. INTRODUCTION

SCOPE OF THE DOCUMENT

This document 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 Articles 5(8) and 17(8) of Regulation (EC) No 1829/2003. Guidance is provided on the drawing up of Annex IIIA of the Directive 2001/18/EC[6] on the deliberate release into the environment of GMMs, on the preparation of an environmental risk assessment (ERA) as stated in Annex II paragraph D.1, and on the establishment of an environmental monitoring plan according to Annex VII of that Directive.

For the purpose of this guidance document, the GMMs covered include archaea, bacteria and eukarya. Eukarya include filamentous fungi, yeasts, protozoa and microalgae[7]. This document does not cover the use of tissue cultures of plant or animal cells[8], or viruses or viroids. In the case of 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.

With regard to products obtained by fermentation of GMMs which do not fall under Regulation (EC) No 1829/2003[9], this guidance covers the assessment of the final product to be used as food or feed for placing on the market, while taking into account the characteristics of the GMM, but does not cover the production process as such that is performed under containment according to Directive 2009/41/EC[10]. Depending on the category and scope of the product, its characterisation and safety assessment will be further undertaken according to relevant legislation for which different guidance documents or guidelines apply.

GMMs used as plant protection products or biocides, fall within the scope of the Directive 2001/18/EC and such microorganisms are not considered food or feed and, therefore, are not covered by this guidance document. This guidance does not cover the deliberate release into the environment of GMMs for any other purpose than for placing on the market (Directive 2001/18/EC). This exclusion covers releases for experimental purposes and for research; such releases fall under Part B of Directive 2001/18/EC. This guidance does not cover issues related to risk management (traceability, labelling, etc.). Socioeconomic and ethical issues are also outside of the scope of this guidance.

The EU Regulations, Directives and Decisions published in the Official Journal of the European Union 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. Questions with regard to the interpretation of the relevant EU legislation should, therefore, be addressed to the European Commission.

[6] Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. Official Journal of the European Communities L106: 1-38.

[7] For further information on taxonomy, please refer to the OECD Guidance Document on the use of taxonomy in Risk Assessment of Microorganisms: Bacteria (OECD, 2010a).

[8] Directive 2009/41/EC defines microorganisms as “any microbiological entity, cellular or non-cellular, capable of replication or of transferring genetic material, including viruses, viroids, animal and plant cells in culture.

[9] For more information, see the Report from the Commission to the Council and the European Parliament on the implementation of Regulation (EC) No 1829/2003 of the European Parliament and of the Council on genetically modified food and feed (COM(2006) 626 final).

[10] Directive 2009/41/EC of the European Parliament and of the Council of 6 May 2009 on the contained use of genetically modified micro-organisms. OJ L 125, 21.05.2009, p. 75-97.

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

The objective of the risk assessment is, on a case by case basis, to identify and evaluate potential adverse effects of the GMM, either direct or indirect, immediate or delayed, on human and animal health and the environment, linked to placing GMMs and/or their products for food and feed use on the market. The comparative approach, considering closely related microorganisms or their products with a history of safe use, is a key general principle in risk assessment of GMMs.

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.

This document is targeted to the principles of risk assessment, which is a scientific exercise. An extensive overview of risk assessment procedures is provided by the Scientific Committee of EFSA (EFSA, 2009a) and for ERA by the EU (EC, 2000). The information required to structure the risk assessment process of GMMs and their products is further detailed in Chapter III (Sections B-C) 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.

DEFINITIONS IN RISK ASSESSMENT

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

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 humans, animals 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.

Hazard identification. In hazard identification, potential adverse effects (hazards) are identified on the basis of knowledge about the characteristics of the recipient microorganism, knowledge about the function that the introduced traits have in the donor organism, knowledge about the way the newly acquired traits interact with the physiology of the recipient microorganism, and the anticipated interaction of the GMO with the receiving environment.

Whenever an appropriate comparator is available, hazard identification should be focused on the identification of differences between the GMM and the comparator (see below). 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.

[11] Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p. 1 24.

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). The differences identified during the hazard identification step should be assessed according to Chapter III, Section B.2.4.

Exposure assessment. 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.

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.

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.

CATEGORISATION OF THE GMMS AND THEIR 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 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 microbial food cultures like probiotics or dairy starters at the other. Four product categories are distinguished depending on the nature of the product and resulting information required according to the present guidance document (see Figure 1 and Table 1 for different requirements):

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

Category 2: Complex products in which both GMMs and newly introduced genes are no longer present (e.g. cell extracts, most enzyme preparations);

Category 3: Products derived from GMMs in which GMMs capable of multiplication or of transferring 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 multiplication or of transferring genes (e.g. live starter cultures for fermented foods and feed).

The information requirements described in Chapter III, Section B.1. should be provided for products belonging to all categories, unless otherwise specified (Table 1). In addition, depending on the category and scope of the product, its characterisation and safety assessment should be undertaken according to relevant legislation and/or Guidance Documents or Guidelines:

- Amino acids and enzymes used as feed additives belonging to Categories 1 and 2,

respectively, and microbial feed additives belonging to Category 4 are assessed according to the Commission Regulation (EC) No 429/2008[12] 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. For these applications, Chapter III, Sections B.2.2. and B.4.1. (Categories 1 and 2); or Chapter III, Section B.4.3. (Category 4) of this guidance will apply. According to the nature and use of the product, relevant guidance document(s) of the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) are also applicable (EFSA, online).

- Biomasses used as feed materials belonging to Category 3 are assessed according to the Regulation (EC) No 1829/2003. For these applications, Chapter III, Sections B.2.2. and B.4.2. of this guidance will apply. Applicants should also follow the Guidance on the assessment of microbial biomasses for use in animal nutrition (EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), 2011).

- Food enzymes belonging to Category 2 fall under the scope of Regulation (EC) No 1332/2008[13] and Regulation (EC) No 1331/2008[14]. For these applications, Chapter III, Sections B.2.2. and B.4.1. of this guidance will apply. 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).

- Food additives belonging to Categories 1 or 2 fall under the scope of Regulation (EC) No 1333/2008[15] and Regulation (EC) No 1331/2008. For these applications, Chapter III, Sections B.2.2. and B.4.1. of this guidance will apply. Applications should follow the Guidance on Submissions for Food additive Evaluations by the Scientific Committee on Food (EC, 2001).[16]

- Food flavourings belonging to Categories 1 or 2 fall under the scope of Regulation (EC) No 1334/2008[17] and Regulation (EC) No 1331/2008. For these applications, Chapter III, Sections B.2.2. and B.4.1. of this guidance will apply. 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).

- Products other than the ones mentioned above, consisting of, containing, or produced from GMMs should be assessed according to the principles laid down in Regulation

[12] Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives. OJ L 133, 22.05.2008, p. 1–65.

[13] Regulation (EC) No 1332/2008 of the European Parliament and of the Council of 16 December 2008 on food enzymes and amending Council Directive 83/417/EEC, Council Regulation (EC) No 1493/1999, Directive 2000/13/EC, Council Directive 2001/112/EC and Regulation (EC) No 258/97. OJ L 354, 31.12.2008, p. 7–15.

[14] Regulation (EC) No 1331/2008 of the European Parliament and of the Council of 16 December 2008 establishing a common authorisation procedure for food additives, food enzymes and food flavourings. OJ L 354, 31.12.2008, p. 1–6.

[15] Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. OJ L 354, 31.12.2008, p. 16–33.

[16] 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.

[17] Regulation (EC) No 1334/2008 of the European Parliament and of the Council of 16 December 2008 on flavourings and certain food ingredients with flavouring properties for use in and on foods and amending Council Regulation (EEC) No 1601/91, Regulations (EC) No 2232/96 and (EC) No 110/2008 and Directive 2000/13/EC. OJ L 354, 31.12.2008, p. 34–50.

(EC) No 1829/2003 and should follow Chapter III, Sections B.2.1. to B.4. of this guidance, For products under Category 4, Chapter III, parts of Section B.2.2. do not apply (B.2.2.1. to B.2.2.3.).

COMPARATIVE APPROACH

The comparative approach is used as an internationally accepted baseline for the assessment of risks in the frame of human and animal health. Regulation (EC) No 1829/2003 defines the comparator (conventional counterpart) as a similar food or feed produced without the help of genetic modification (as defined in Directive 2001/18/EC) and for which there is a well-established history of safe use. According to Directive 2001/18/EC, the general principle to be followed when performing ERA is that identified characteristics of the GMO and its use which have the potential to cause adverse effects should be compared to those presented by the non-modified organism from which it is derived and its use under corresponding situations. This comparison will assist in identifying the particular potential adverse effects arising from the genetic modification. The most appropriate comparator would thus be the non-modified parental or recipient strain that is identical except for the introduced trait(s). However, in case of GMMs the parental microorganism has often gone through a number of genetic modification steps before the intended modifications that are subjects of the assessment. In those cases, a key consideration is whether the comparator is a strain which has previously been evaluated for safety. Along these lines, the *Codex Alimentarius* (Codex Alimentarius, 2003b) defines the comparator (conventional counterpart) as "a microorganism/strain with a known history of safe use in producing and/or processing the food and related to the recombinant-DNA strain; or food produced using the traditional food production microorganisms for which there is experience of establishing safety based on common use in food production".

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 also 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, 2007a). 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 antimicrobial[18] resistance or production of toxins). The list of QPS organisms is reviewed annually and updated (EFSA Panel on Biological Hazards (BIOHAZ), 2010). Whenever possible, the comparator should have a QPS status. 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 microorganism has a QPS status, the risk assessment can focus on the changes introduced (intended and unintended) 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 indicate clearly the relationship and differences between the recipient and the ancestral strains assessed for

[18] 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.

safety. The safety assessment of the GMM may then focus on those differences, together with those introduced during the development of the final GMM.

When the recipient strain does not have a history of safe use, the choice of a different strain of the same species or a phylogenetically close relative as comparator must be justified. All the available information should be provided and evaluated on a case-by-case basis.

Where no comparator can be identified for the GMM and/or its product, a comparative safety assessment cannot be made and a comprehensive safety assessment should be carried out according to the principles laid out in Chapter III, Section B.2.4.

INTENDED AND UNINTENDED EFFECTS

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

Unintended effects are changes other than the intended changes in the GMM resulting from its genetic modification. Some but not all unintended effects might be predicted or explained in terms of current knowledge of biology and of the integration of metabolic pathways.

Intended and predicted unintended effects should be analysed based on the most appropriate methodology. Overall data on different levels, such as molecular characterisation, comparative compositional analysis, phenotypic characteristics, etc. should be used to detect unintended effects. Data on one level can give indications on what should be especially considered on another level. Unintended effects are addressed in the safety and nutritional assessment of the GMM and/or their products as described in this guidance document.

III. INFORMATION REQUIRED IN APPLICATIONS FOR GMMS AND/OR THEIR PRODUCTS

This guidance was developed to support applicants in preparation and presentation of applications submitted under Regulation (EC) No 1829/2003, and also under Regulations (EC) No 1332/2008, 1333/2008, 1334/2008 and 1831/2003 when products involve GMMs. 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. The structure of 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 (see Appendix).

In the case of GMMs obtained by self-cloning and their products for food and feed use, 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 each single case. Unless otherwise specified, the applicant is advised to refer to Chapter II (pages 7- 9), Figure 1 (page 38), and Table 1 (page 40) for an indication of which data must be supplied for applications belonging to each category. Reasons must be given for the omission of data not considered to be relevant.

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:

- the name and address of the applicant (company or institute);
- the name, qualification and experience of the responsible scientist(s) and contact details of the person responsible for all dealings with EFSA;
- the title of the project;
- the scope of the application considering the categorisation as defined in Chapter II, Section “Categorisation of risk assessment of GMMs and their derived products”;
- the 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;
- a short description of the method of production and manufacturing.
- the conditions for placing on the market of the food(s) or feed(s) produced from the GMM, including specific conditions for use and handling, when appropriate.

B. HAZARD IDENTIFICATION, HAZARD CHARACTERISATION AND EXPOSURE ASSESMENT

1. Information relating to the GMM

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

Comprehensive information relating to the recipient/parental strain should be provided:

- to identify the need for specific analyses e.g. the known occurrence in related microorganisms (e.g. within 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. All previous modifications, including mutagenesis and selection, undertaken to create the recipient, should be described. If available, the QPS status can be used to meet the information requirements. Information should 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, (g) commercial name.

The taxonomical identification of the parental microorganism is important for the hazard identification 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 (2003) 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 Centre 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

Relevant 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. Relevant genotypic markers refer to e.g. auxotrophic mutations and the identity of genes coding for antimicrobial resistance. Antimicrobial resistance data should be provided in accordance with the most recent EFSA guidance (EFSA, 2007b; EFSA, 2008; EFSA, 2010).

1.1.3. Degree of relatedness between recipient and donor(s)

The relatedness between the recipient and donor(s) should be described, when appropriate (such as when donor and recipient strains are close relatives e.g. belong to the same species).

1.1.4. Description of identification and detection techniques

The technique used for identification of the recipient/parental organism, as well as the methods to detect the organism 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.

1.1.5. Source and natural habitat of the parental microorganism

Information should be provided on the source from which the parental strain has been isolated. Furthermore, if available, 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) should be provided.

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

The inherent capability of the parental microorganism to exchange genes may influence the potential for horizontal gene transfer (OECD, 2010b). 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 presence of plasmids and their host range (specificity);
- the presence of genes that confer resistance/tolerance (e.g. to antimicrobials, heavy metals, toxins), especially if they are associated with mobile genetic elements (e.g. conjugative transposons, prophages, integrons and/or sex/mating factors).

1.1.7. Information on the genetic stability of the recipient microorganism

The genetic stability of the recipient/parental microorganism should be verified to confirm its identity, phenotypic and genotypic characteristics. 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 must be established.

1.1.8. Pathogenicity, ecological and physiological traits

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 EU 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/EC[19]).

b) Information relating to pathogenicity, infectivity, toxigenicity, virulence, and allergenicity should be provided, as appropriate. The whole human population, including vulnerable groups such as immunocompromised individuals, allergic people, infants, pregnant women and the elderly, should be taken into consideration:

   - Information should be provided on the history of 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.
   - 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

[19] Directive 2000/54/EC of the European Parliament and of the Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work. Official Journal of the European Communities L262, 21–45.

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.

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

- The presence of antimicrobial resistance genes introduced into cells should be analysed using appropriate methods, such as DNA sequencing or Southern analysis. Phenotypic antimicrobial resistance data have to be provided following 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 (e.g. integrons, transposons, plasmids).

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.

1.1.9. Description of history of use

Documented information on 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, animal or environmental 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 antimicrobial resistance genes). With a non-QPS organism the previous history of use of the parental strain of the GMM should be presented.

1.1.10. 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.

On a case-by-case basis, particularly when the sequence is synthetically constructed or obtained from environmental samples, a bioinformatic analysis of the DNA sequence obtained to search for homology with known genes in order to find the closest known related sequences may be requested. An updated database search and a discussion of the best hits should then be provided.

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 1.1.5 and 1.1.8. 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;

- DNA sequence and a physical map of the functional elements;

- derived amino acid sequence(s) and function(s) of the encoded protein(s) and, on a case-by-case basis, the role played in the metabolism and substrate specificity.

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 or RT-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 nucleic acid extraction and amplification/cloning procedure;

- rationale and strategy for the selection of the nucleic acid sequence(s);

- similarities with sequences and function in taxonomically defined organisms;

- DNA sequence and a physical map of the functional elements;

- derived amino acid sequence(s) and function(s) of the encoded protein(s) and, on a case-by-case basis, 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;
- it should be indicated whether the introduced DNA remains in the vector[20] or is inserted into the chromosome(s) or into plasmid(s) or, for eukaryotic microorganisms, into DNA of organelles (mitochondria, chloroplasts, etc.);
- a description of the sequences actually inserted, replaced or modified, and in the case of insertions, the copy number of the inserts should be provided and accompanied by experimental data obtained by e.g. Southern analysis, quantitative real time PCR, or a combination of such methods. In case of inserted genes, the unique name should be provided;
- 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 the inserted DNA is located in a mobile genetic element 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 (e.g. by quantitative real time PCR);
- information relating to the host range;
- the frequency of mobilisation and the transfer capacity of the inserted vector(s) used for

[20] "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.

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

The applicant is requested to deposit the GMM in a recognised culture collection and to provide the deposition number.

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 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 purpose of the genetic modification should be described. The intended changes in the phenotype and metabolism of the microorganism should be described. A description of all traits and changes resulting from the genetic modification is required.

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 batches, representative for its production and intended use. At least three independent batches are considered an appropriate number for such studies. 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 expression level of the inserted genetic material should be determined. The methods used for expression analysis and their sensitivity and specificity should be described.

- The conditions under which expression levels were analysed should be provided. The information on expression levels should be derived using representative, at least three, independent 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 inserted DNA encodes an enzyme, its activity and mode of action 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 mobile genetic 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 other microorganisms as a consequence of unintentional gene transfer.

1.4.7. History of previous uses or environmental releases of the GMM, when 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). Such changes could result in both intended and potentially unintended effects that may affect human or animal health. The following information should, therefore, be provided:

- 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;
- 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 are required;
- if the GMM remains viable (Category 4), 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 genetic modification may enhance the ability of the GMM to persist in the gastrointestinal tract (e.g. increased pH tolerance);
- any impact that the GMM may have on the microbiota of the human or animal gastrointestinal tract.

2. Information relating to the product (including cases when the GMM itself is the product)

Applicants should indicate to which of the four categories of GMMs or their products the new product belongs, as defined in Chapter II. Depending of the category and scope, the characterisation (specification) and safety assessment will be undertaken according to relevant legislation and/or Guidance Documents or Guidelines (See Chapter II).

For Categories 1, 2 and 3, it will be necessary to understand the processes by which the GMM has been removed or inactivated in the product. It should be confirmed that the product does not contain viable but non-cultivable cells (VBNC) or spores. For Categories 1 and 2, it should be demonstrated that no recombinant genes remain in the product.

Information relating to the GMM and/or their products should include a description of their main characteristics and their 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.

Information should be provided on the GMM and/or the derived product, and also on the effects of the GMM or the derived product on the food containing them. 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 for the safety evaluation.

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

Depending on the category, information relating to the preparation of the product should be provided and/or the description of techniques used to remove/inactivate GMM cells and recombinant genes should be included (Table 1).

2.2.1. Demonstration of the absence of the GMM in the product

Removal of the GMM is required in products belonging to Categories 1 and 2. Products belonging to Category 3 may also be prepared by removing the GMM. The technique used to remove microbial cells in the course of the product preparation process should be detailed and the absence of the GMM from the product should be demonstrated.

- 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 organism.

- It is recommended that at least three independent batches of product preparations are sampled, each analysed in triplicate. Alternative analysing strategies should be justified. A reliable sampling method should be chosen and documented, taking into account the possible heterogeneity of the product. For example, an analytical sample can be prepared by pooling at least 10 different individual samples taken from the same preparation batch. Samples should be taken from at least a pilot scale production and

information on the scale of production should be provided.

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

2.2.2. Information on the inactivation of the GMM cells and evaluation 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. Products belonging to Category 2 may also be prepared by inactivating the GMM. 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 (including spores) 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 exposed humans and animals. Therefore, the possible presence of physically intact cells, viable but non-cultivable (VBNC) cells, and stressed cells should be determined. The VBNC state is well described for some bacteria and may also exist in certain yeasts. The potential hazard 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 human- or animal-associated 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.

- The absence of viable GMM cells should be verified by means of a method targeted to the detection of the viable GMM. In case when there is an extensive background microbiota, appropriate selective culture media may be used. The methods should be described and the levels of detection documented.

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

- If the GMM can form spores, their possible presence should be analysed by using germination procedures adapted to the organisms and subsequent culturing.

- If appropriate for the bacterial or yeast species of concern, the possible presence of VBNC should be analysed by culture-independent 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).

- Depending on the characteristics of the microorganism, the possible presence of physically intact dead cells should be analysed by culture-independent techniques; epifluorescence techniques (microscopy, flow cytometry) may be used.

- It is recommended that at least three independent batches of product preparations are sampled, each analysed in triplicate. Alternative analysing strategies should be justified. 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.4.). All the methods should be documented in detail.

- All DNA present in the product should be extracted. Therefore, a cell lysis step must be included in the protocol to extract DNA from products belonging to Categories 2 or 3. Special attention should be given to the detection of DNA present in microorganisms that are resistant to cell lysis, like those capable of forming spores. To verify the efficacy of the lysis step, intact cells of the GMM must be added in different dilutions before DNA extraction as a positive control.

- 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 (e.g. antimicrobial resistance genes, virulence genes, genes encoding toxic compounds), 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

The following Section applies to food consisting of, containing, or produced from GMMs falling under Regulation (EC) No 1829/2003.

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, when applicable, should be described. Any other potential uses should also be specified.

2.3.3. Composition

Depending on the category and use, qualitative and, when appropriate, quantitative composition of the GMM and/or its product should be provided. The extent of batch-to-batch variation should be determined. At least three representative independent 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, on a case-by case basis, for products belonging to Category 2, the analysis should include the relevant nutrients, antinutrients, and other metabolites typical of the product (organic acids, alcohols, flavour components, etc.) and, on a case-by-case basis, specific impurities. When relevant conventional products exist, they should be used as comparators for the corresponding GMM and/or its product.

2.3.4. Physical properties

Depending on the category and use of the product, the applicant should describe the physical state (liquid, solid) of the product. The most appropriate physical properties (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 indicated.

2.3.5. Technological properties

Depending on the category (Table 1), the technological attributes of the product should be specified for its intended use. The stability of the product, or activity, and the shelf-life should be defined for the conditions in which it is to be used, when appropriate. Methods used for the determination of these properties, their accuracy, reliability and efficiency should be described.

2.4. Considerations of the GMM and/or its product for human health

The following Section applies to food consisting of, containing, or produced from GMMs falling under Regulation (EC) No 1829/2003. Considerations are mainly relevant to products belonging to categories 3 and 4, but in some cases also for products of Category 2. The first step in the hazard characterisation should be the comparison of the GMM and/or its product with its appropriate comparator (see Chapter II, Section “Comparative approach”). This comparison should focus principally on the differences in relevant metabolites between the GMM and its appropriate comparator growing in the same matrix and in the same food 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 should often, in practice, be combined with the compositional analysis of the food 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 food products may still be used as comparators to identify possible compositional changes and to assess their safety implications.

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

Genes inserted in a GMM and their encoded proteins should be evaluated for their potential impact on human health, and the evaluation should include consideration of the potential for the GMM to transfer genetic material to other organisms and its capacity 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.4.).

2.4.1. Toxicology

The following Section applies to food consisting of, containing, or produced from GMMs falling under Regulation (EC) No 1829/2003. Considerations are mainly relevant to products belonging to categories 3 and 4, but in some cases also for products of Category 2. The toxicological impact of any

changes in the GMM and/or its 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 toxicological assessment should also focus on the metabolites produced by the GMM during the fermentation process and released into the food. The toxicological assessment of the 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[21] 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.

2.4.1.1. Evaluation of proteins expressed by the introduced genes

Proteins expressed by the 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. When it has been established that the proteins have been safely consumed (Constable et al., 2007), 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 introduced genes in the GMM must be demonstrated.

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

- molecular and biochemical characterisation of the protein(s) expressed by the introduced gene(s), including the sequence, molecular weight, and a description of the function (see Section B.1.4.4.);
- a search for similarity 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

[21] Directive 2004/10/EC of the European Parliament and of the Council of 11 February 2004 on the harmonisation of laws, regulations and administrative provisions relating to the application of the principles of good laboratory practice and the verification of their applications for tests on chemical substances. OJ L 50, 20.2.2004, p. 44–59.

concern, a repeated dose animal toxicity study with the protein expressed by the introduced gene or other appropriate studies should be considered. Depending on the outcome of these studies, further investigations may be required.

2.4.1.2. Evaluation of constituents other than proteins

New constituents other than proteins, as well as any anticipated changes in specific metabolic pathways due to the modification, 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 (EC, 2001) may be needed.

If due to the modification of specific metabolic pathways, the levels of naturally occurring metabolites have been changed an evaluation based on the knowledge of the physiological function and/or toxic properties of these constituents, as well as the anticipated changes in intake levels should be carried out. The result of this assessment would determine if, and to what extent, toxicological tests are required.

2.4.1.3. Evaluation of the product (including cases when the GMM itself is the product)

Evaluation of the GMM

The hazard characterisation of a GMM is primarily based on the molecular characterisation of the genetic modification, a comparative compositional analysis in relation to an appropriate comparator, and on the assessment of the identified intended and unintended effects (Section B.1.4.8.). When these types of analyses indicate a need (see below) to perform an animal study to check whether the GMM is as safe as its comparator, a 90-day rodent feeding study with the GMM should be performed.

If there are any indications of unintended effects based on the preceding molecular characterisation of the genetic modification and/or compositional analysis, toxicological testing of the GMM should be undertaken. Toxicological testing of the GMM should also be performed when no conventional counterpart exists (e.g. when the composition of the GMM is modified substantially, as may be the case with extensive genetic modifications targeted at specific alterations in the metabolism leading to substantial compositional changes).

The design of the toxicity study with the GMM should be adapted from the OECD 90-day rodent toxicity study, Guideline 408 (OECD, 1998). Normally a minimum of two test dose levels and a negative control is used. The highest dose level should be the maximum achievable one, without causing nutritional imbalance, and the lowest dose level should contain at least an amount of the test food equivalent to that consumed by humans. Stability of test diets and nutritional equivalence between control and test diets are important aspects to consider.

Depending on the outcome of the 90-day rodent feeding study or if other information on the GMM indicates the need for additional testing further specific investigations may be required.

In cases where the molecular and compositional analyses have demonstrated equivalence between the GMM and the respective comparator, except for the inserted trait(s), and have not indicated that unintended effects may occur, the performance of animal feeding trials with rodents or other (target) animal species is of little additional value if any, and is, therefore, not deemed necessary.

For GMMs under Categories 3 and 4, particular attention should be paid to interaction(s) with the gut microbiota and the evaluation of any effect on the digestive physiology and human immune response. This may require specific testing including studies in humans. If the GMM remains viable in the final food, information on the viability and residence time of the GMM in the gastrointestinal tract should

be provided; this is particularly important if the genetic modification may enhance the ability of the GMM to persist in the gastrointestinal tract (e.g. increased pH tolerance).

Evaluation of the product

In cases when the composition of the product has been substantially changed by the GMM with respect to the comparator or if there is no appropriate comparator, the final product should also be tested toxicologically. The products may represent cell extracts or biomasses or foods prepared by GMM-associated processes.

Due to the wide variety of possible products, the requirements for toxicological testing should be selected case by case and justified. When applicable, the 90-day rodent feeding study, as outlined in Section B.2.4.1.3., is a recommended approach. The lowest dose should correspond to the level of expected human consumption. The highest dose level should be the maximum achievable one, without causing nutritional imbalance. If there is no appropriate comparator, the choice of control(s) should be justified taking into account the nature of the test product.

It is recognised that testing of whole foods may not in all cases be feasible. In those cases the relevant fraction of the food should be selected for testing and the selection should be justified.

Depending on the outcome of the toxicological testing, or if other information on the product indicates the need of additional testing, further investigations may be required.

2.4.2. Allergenicity

2.4.2.1. Introduction

The following Section applies to food consisting of, containing, or produced from GMMs falling under Regulation (EC) No 1829/2003. Considerations are mainly relevant to products belonging to Categories 3 and 4, but in some cases also for products of Category 2.

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. possible coeliac 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 GMMs and their products as well as gluten intolerance.

The food constituents that are responsible for allergenicity are in nearly all cases proteins or peptides. In a few cases low molecular weight substances 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..

The integrated process which is described below covers both food and respiratory allergy risk in humans. When appropriate, allergy in animals should similarly be considered.

2.4.2.2. Proteins expressed by the introduced genes

At present, there is no definitive test that can predict an IgE-mediated allergic response in humans to a protein expressed by an 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 Intergovernmental Task Force on Foods Derived from Biotechnology (Codex Alimentarius, 2003a), an integrated, stepwise, case-by-case approach, as described below, should be used in the assessment of possible allergenicity of proteins expressed by the 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 introduced genes for a possible role in the elicitation of gluten-sensitive enteropathy.

- Amino acid sequence homology comparison between the protein expressed by the 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 peptide fragments in the amino acid sequence of the protein. The number of 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 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.

    - If the source of the introduced DNA sequence is considered allergenic, but no sequence homology of the protein expressed by the 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 introduced gene may be considered very likely to be allergenic. If no IgE binding is observed, the protein expressed by the introduced gene should still undergo pepsin resistance tests and additional testing.

    - 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 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. In some cases, testing against a panel of sera with reactivity against organisms related to the source organism (which may express similar allergens) may be performed ('targeted serum screening').

- Resistance to pepsin digestion. Resistance 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 weight-of-evidence risk evaluation. The use of well standardised methodology, including appropriate allergenic and non-allergenic controls, is essential (EFSA Panel on Genetically Modified Organisms (GMO), 2010). 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 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 introduced gene(s) may 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, may be considered by additional in vitro digestibility tests more closely simulating actual gastric conditions (pH variation, etc.). 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.

2.4.2.3. Evaluation of allergenicity of the product (including cases when the GMM itself is the product)

The possibility of increased overall allergenicity of the GMM and/or its product should be considered (e.g. expression of allergens, including endogenous allergens).

The approach should be selected on a case-by-case basis depending on the available information on the allergenic potential of the host. Established methods for allergen quantification should be used. In addition, proteomics and protein profiling techniques may be used in addition to human and animal serum or cell-based assays.

2.4.2.4. 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 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.

2.4.3. Nutritional assessment

The following Section applies to food consisting of, containing, or produced from GMMs falling under Regulation (EC) No 1829/2003. Considerations are mainly relevant to products belonging to Categories 3 and 4, but in some cases also for products of Category 2. Identification of compositional changes in key nutrients and antinutrients is the starting point for the nutritional assessment of a GMM

and/or its 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 GMMs and/or their products. In particular, if the GMM and/or its product is targeted for a certain consumer group (e.g. infants, the elderly, individuals with food allergy) or for some special purpose (e.g. weight control, functional food, dietary supplement), the effects on the overall nutrition should be carefully assessed.

- When compositional equivalence of the GMM and/or its 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 GMM and/or its 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 GMM and/or its product, taking into account any possible replacement of existing food, will be required and the nutritional consequences should be assessed 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. Exposure assessment/characterisation related to food and feed consumption

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 D.).

4. Potential environmental impact of GMMs and their products

The authorisation of GMMs and their products requires an ERA with the objective of identifying their potential environmental damage and the likelihood of its occurrence. The ERA of Category 4 GMM shall be in line with the requirements laid down in Directive 2001/18/EC (including Annex II). 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[22]. 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 gastrointestinal tract, terrestrial ecosystems where 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

[22] Directive 2004/35/CE of the European Parliament and of the Council of 21 April 2004 on environmental liability with regard to the prevention and remedying of environmental damage. OJ L 143, 30.04.2004, p. 56-75.

and relevant routes of entry are identified, the possibility of adverse health effects should be evaluated. The ERA must also consider potential cumulative long-term effects associated with the interaction with biotic and abiotic components of the environment. 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.

4.1. Evaluation of products belonging to Categories 1 and 2

ERA of Categories 1 and 2 concerns demonstration of absence of viable GMMs or their recombinant DNA in the products. Guidance to demonstrate absence of viable GMMs and recombinant DNA is provided in Sections B.2.2.1. to B.2.2.3. Environmental exposure of the GMM is negligible provided that no viable GMMs and recombinant genes originating from them are present. Requirements to assess environmental effects e.g. ecotoxicological properties of products themselves falling under these categories are legislated elsewhere (see, pages 7- 9) and not covered here.

4.2. Evaluation of products belonging to Category 3

Since the lack of viability (potential to replicate) of the GMM must have been demonstrated (see Section B.2.2.2.), the ERA 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. In more detail, information on the following information should be provided:

- 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 ERA 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 varies between specific environments and, therefore, requires considerations about the suspected density of microbial cells and presence of bacteria

known to develop competence for natural transformation. Most recent information about bacterial species capable of natural transformation and their requirements to develop competence should be retrieved from the literature or databases. Starting points for searches may be Lorenz and Wackernagel (1994) or Dubnau (1999).

- 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, e.g. biogeochemical cycles.

4.3. Evaluation of products belonging to Category 4

This category requires the most detailed assessment. This assessment needs to consider whether the GMM is capable to survive (persist) and proliferate in specific environments, the possibility that presence of 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.

Environmental hazard characterisation 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) potential 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 estimated.

- The potential of the GMM to survive (persist) and proliferate in receiving environments. Information is needed concerning 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 (e.g. pH and temperature range, 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 viable but non-cultivable (VBNC) state.

- Possible interactions of GMMs with their abiotic and biotic environments 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 determined 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 has the potential to cause adverse effects on other microorganisms or the alteration of key 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 in more detail 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).

- Consideration of mechanisms which may allow the GMM to transfer recombinant DNA to environmental microorganisms:

    - 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 remains a highly efficient and likely gene transfer mechanism among bacteria (Courvalin, 2008; EFSA, 2009c), GMMs with plasmids and/or conjugative transposons 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 hazard characterisation of GMMs that have close relatives in a receiving environment(e.g. *Enterobacteriaceae* for *E. coli*). 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.

    - Eukaryotes. For fungi, protozoa and microalgae mating is the process by which gene transfer usually 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.

- The 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 phages released by the GMM, or the presence of microorganisms competent for 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 a mycorrhizal fungus, 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.

- The 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.

- The effects of GMMs on animals. When appropriate, expected, unintended exposure to 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. RISK CHARACTERISATION

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 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, the 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 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 or abiotic components can take place. It is recognised that an ERA 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. In cases when GMMs or recombinant DNA repeatedly enter the environment over a long period, potential cumulative long-term effects associated with the interaction with biotic and abiotic components of the environment should be considered. Experience with the introductions of other microorganisms or GMMs of similar properties (species, ecophysiology, genetic modification) and an understanding of the stability and resilience of the relevant ecosystems can be helpful for estimating the environmental impact.

The assessment of environmental impacts is not finished with the pre-market evaluation of a GMM or their product (as relevant for this guidance document). It is a crucial component of the required post-market environmental 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. product labelling for post-market monitoring). If management strategies are proposed, these should be an objective of a new assessment to conclude on their efficacy and of case specific monitoring to verify the expected efficacy of the strategies or measures.

D. POST-MARKET MONITORING REGARDING USE OF THE GMM AND/OR ITS PRODUCT FOR FOOD OR FEED

Where appropriate a Post Market Monitoring (PMM) programme should be performed for food and feed derived from GMMs. PMM does not substitute a thorough pre-marketing toxicological testing programme, rather complements it in order to confirm the pre-market risk assessment. It may increase the probability of detecting rare unintended effects. Therefore the PMM for food and feed should be designed to generate reliable and validated flow of information between the different stakeholders which may relate consumption of food and feed derived from GMMs to any (adverse) effect on human and animal health.

As pre-market risk assessment studies cannot fully reproduce the diversity of the populations who will consume the marketed product, the possibility that unpredicted side effects may occur in some individuals, such as those with certain disease states, those with particular genetic/physiological characteristics or those who consume the products at high levels remains. Indeed, risk assessment also relies on an estimate of exposure to the food, which is variable and subject to uncertainty before the food is marketed. A PMM should, therefore, address the following questions: is the product use as predicted/recommended? Are known effects and side-effects as predicted? Does the product induce unexpected side effects? (Wal *et al.*, 2003)

PMM should be required only in specific cases, such as foods with altered nutritional composition and modified nutritional value and/or with specific health claims. A similar approach can apply to feed with altered nutritional characteristics.

E. POST-MARKET ENVIRONMENTAL MONITORING (PMEM)

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 D. 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 GMMs, and their management to human health or the environment, after the GMM 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[23] (see also ACRE, 2004; Wilhelm et al., 2003).

A PMEM plan 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, but not those of categories 1, 2 and 3, 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 E.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 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 aims: (1) to study any possible adverse environmental effects of the GMM identified (anticipated) in the ERA, and (2) to identify the occurrence of unforeseen adverse environmental effects of the GMM which were not anticipated in the ERA. Case-specific monitoring should be carried out after placing on the market, when potential adverse effects or significant levels of critical uncertainty linked to the GMM have been identified in the ERA, in order to inform the ERA further. Consequently, case-specific monitoring is required when there is a need to verify the risk assessment and is not obligatory. By contrast a general surveillance plan must be part of each application. Applicants should clearly state in their risk assessment conclusions the reasons and the scientific evidence why no case-specific monitoring is required. Their arguments should relate to the assumptions applicants have made in the ERA as well as to the lack of adverse effects.

GMM products belonging to Category 3 do not fall under Directive 2001/18(EC) and, therefore, a general surveillance is not required. Nevertheless, if horizontal gene transfer is considered in the ERA to pose a risk case-specific monitoring should be considered as laid out in Annex VII.

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.

[23] Council Decision (2002/811/EC) of 3 October 2002 establishing guidance notes supplementing Annex VII to Directive 2001/18/EC of the European Parliament and of the Council on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. OJ L 280, 18.10.2002, p. 27-36.

2. Case-specific 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 C.). 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 ERA. The scientific approach should be designed in order to test the specific hypothesis of potential adverse effects derived from the ERA. The methods selected, the intervals and extent of sampling should be determined on a case-by-case basis.

3. General surveillance

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:

- an observed effect is unusual;
- an unusual effect is adverse; and
- the adverse effect is associated with the GMM or its use.

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.

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.

If unusual observations on human or animal health and the environment are reported, more focused 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 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 collect 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.

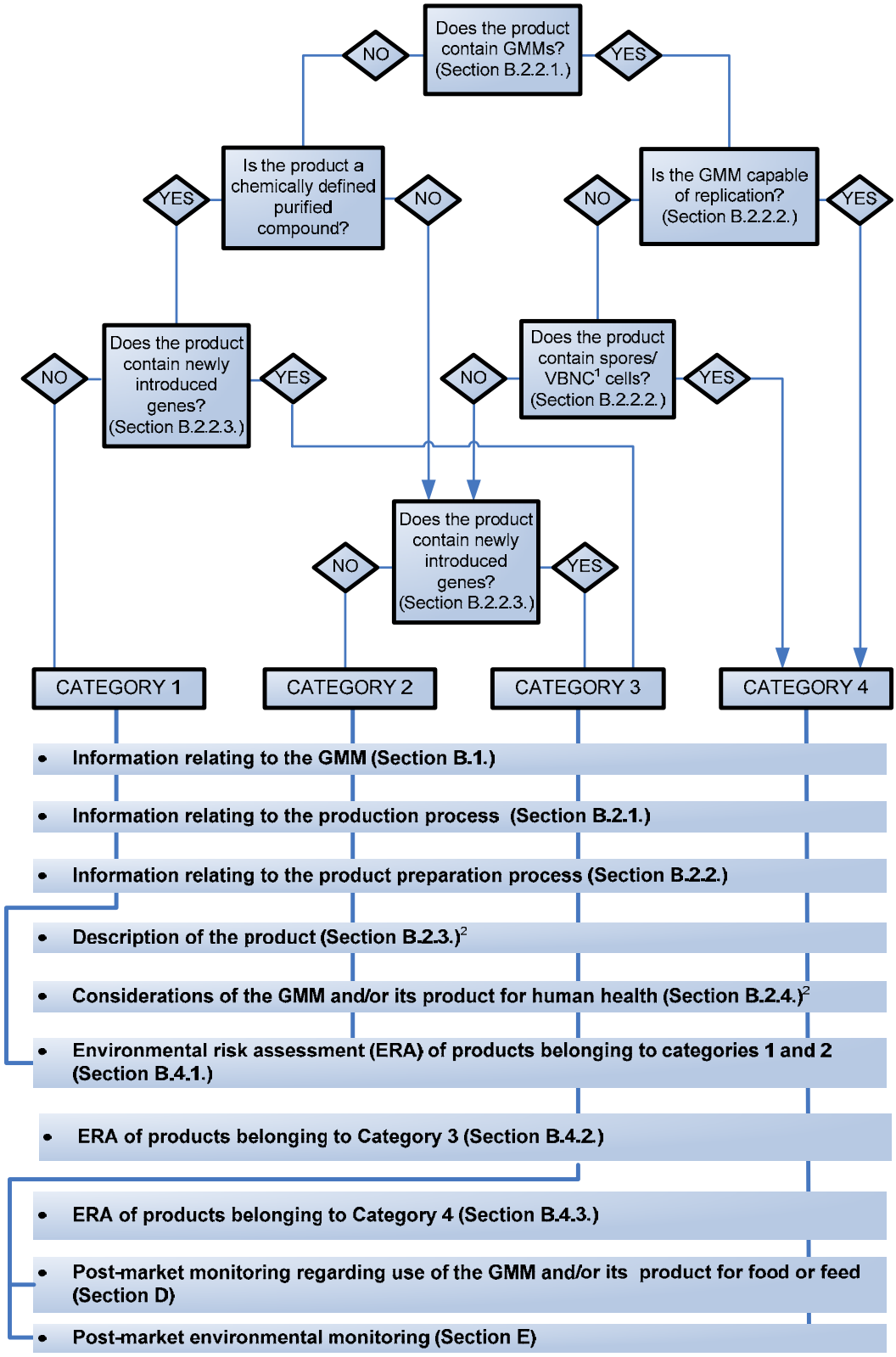
At present there are no suitable large-scale surveillance and monitoring systems suitable for the identification of possible adverse health effects posed by the handling or use of GMMs in humans. Experience in designing surveys and their statistical analysis is available from other established surveillance 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[24] concerning the reporting format.

[24] Commission Decision (2009/770/EC) of 13 October 2009 establishing standard reporting formats for presenting the monitoring results of the deliberate release into the environment of genetically modified organisms, as or in products, for the purpose of placing on the market, pursuant to Directive 2001/18/EC of the European Parliament and of the Council. OJ L 275, 21.10.2009, p. 9–27.

Figure 1: Flow diagram showing the approach to the categorisation of GMM and/or their products and associated risk assessment



[1] Viable But Non-Cultivable.
[2] Depending on the product, these Sections may not be applicable, as they are covered by other relevant guidance and guidelines (see Chapter II, pages 7-9).

F. 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 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 on the market of GMMs and their derived food and feed products

	Category 1 Chemically defined purified compounds and their mixtures in which both GMMs and newly introduced genes have been removed	**Category 2** Complex products in which both GMMs and newly introduced genes are no longer present	**Category 3** Products derived from GMMs in which GMMs capable of multiplication or of transferring genes are not present, but in which newly introduced genes are still present	**Category 4** Products consisting of or containing GMMs capable of multiplication or of transferring genes	**Chapter, Section**
Characteristics of the recipient or parental microorganism					**III. B.1.1.**
1. Scientific name, taxonomy and other names	X	X	X	X	III. B.1.1.1.
2. Phenotypic and genetic markers	X	X	X	X	III. B.1.1.2.
3. Degree of relatedness between recipient and donor(s)			X[a]	X[a]	III. B.1.1.3.
4. Description of identification and detection techniques			X	X	III. B.1.1.4.
5. Source and natural habitat of the recipient microorganism			X[b]	X	III. B.1.1.5.
6. Organisms with which transfer of genetic material is known to occur under natural conditions and presence of indigenous genetic mobile elements			X	X	III. B.1.1.6.

[a] Information not required in case of self-cloning with the same strain.
[b] Information not required if proposed QPS status is authorised.

	Category 1 Chemically defined purified compounds and their mixtures in which both GMMs and newly introduced genes have been removed	Category 2 Complex products in which both GMMs and newly introduced genes are no longer present	Category 3 Products derived from GMMs in which GMMs capable of multiplication or of transferring genes are not present, but in which newly introduced genes are still present	Category 4 Products consisting of or containing GMMs capable of multiplication or of transferring genes	Chapter, Section
7. Information on the genetic stability of the recipient microorganism		X	X	X	III. B.1.1.7.
8. Pathogenicity, ecological and physiological traits		X[b]	X[b]	X	III. B.1.1.8.
9. Description of its history of use		X[b]	X[b]	X[b]	III. B.1.1.9.
10. History of previous genetic modifications	X	X	X	X	III. B.1.1.10.
Characteristics of the origin of the inserted sequences (donor organism(s))[a]					**III. B.1.2.**
1. DNA from defined donor organisms	X	X	X	X	III. B.1.2.1.
2. Synthetic DNA	X	X	X	X	III. B.1.2.2.
3. Nucleic acids directly extracted from environmental samples	X	X	X	X	III. B.1.2.3.
Description of the genetic modification					**III. B.1.3.**
1. Characteristics of the vector	X	X	X	X	III. B.1.3.1.
2. Information relating to the genetic modification	X	X	X	X	III. B.1.3.2.

	Category 1 Chemically defined purified compounds and their mixtures in which both GMMs and newly introduced genes have been removed	**Category 2** Complex products in which both GMMs and newly introduced genes are no longer present	**Category 3** Products derived from GMMs in which GMMs capable of multiplication or of transferring genes are not present, but in which newly introduced genes are still present	**Category 4** Products consisting of or containing GMMs capable of multiplication or of transferring genes	**Chapter, Section**
Information relating to the GMM and comparison of the GMM with an appropriate comparator					**III. B.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	X	X	X	X	III. B.1.4.1.
2. Structure and amount of any vector and/or donor nucleic acid remaining in the GMM	X	X	X	X	III. B.1.4.2.
3. Stability of the genetic traits in the GMM		X	X	X	III. B.1.4.3.
4. Rate and level of expression of the new genetic material and activity of the expressed proteins			X	X	III. B.1.4.4.
5. Description of identification and detection techniques	X	X	X	X	III. B.1.4.5.
6. Information on the ability to transfer genetic material to other organisms			X	X	III. B.1.4.6.
7. History of previous uses or environmental releases of the GMM, when appropriate			X	X	III. B.1.4.7.
8. Safety for humans and animals		X	X	X	III.B.1.4.8.

	Category 1 Chemically defined purified compounds and their mixtures in which both GMMs and newly introduced genes have been removed	Category 2 Complex products in which both GMMs and newly introduced genes are no longer present	Category 3 Products derived from GMMs in which GMMs capable of multiplication or of transferring genes are not present, but in which newly introduced genes are still present	Category 4 Products consisting of or containing GMMs capable of multiplication or of transferring genes	Chapter, Section
Information relating to the production process	X	X	X	X	**III. B.2.1.**
Information relating to the product preparation process					**III. B.2.2.**
1. Demonstration of the absence of the GMM in the product	X	X	X		III. B.2.2.1.
2. Information on the inactivation of the GMM cells and evaluation of the presence of remaining physically intact cells		X	X		III. B.2.2.2.
3. Information on the possible presence of recombinant DNA	X	X	X		III. B.2.2.3.
Description of the product[c]					**III. B.2.3.**
1. Designation of the product		X	X	X	III. B.2.3.1.
2. Intended use and mode of action		X	X	X	III. B.2.3.2.
3. Composition		X	X	X	III. B.2.3.3.
4. Physical properties		X	X	X	III. B.2.3.4.

[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 Chapter II, page 8.).

	Category 1 Chemically defined purified compounds and their mixtures in which both GMMs and newly introduced genes have been removed	Category 2 Complex products in which both GMMs and newly introduced genes are no longer present	Category 3 Products derived from GMMs in which GMMs capable of multiplication or of transferring genes are not present, but in which newly introduced genes are still present	Category 4 Products consisting of or containing GMMs capable of multiplication or of transferring genes	Chapter, Section
5. Technological properties		X	X	X	III. B.2.3.5.
Considerations of the GM product for human health[c]					**III. B.2.4.**
1. Toxicology		X	X	X	III. B.2.4.1.
5. Allergenicity		X	X	X	III. B.2.4.2.
6. Nutritional assessment		X	X	X	III. B.2.4.3.
Evaluation of products belonging to categories 1 and 2	X	X			**III. B.4.1.**
Evaluation of products belonging to Category 3			X		**III. B.4.2.**
Evaluation of products belonging to Category 4				X	**III. B.4.3.**
Post-market monitoring regarding use of the GMM and/or its product for food or feed[c]		X	X	X	**III. D.**
Post-market environmental monitoring (PMEM) of GM products[c]			X	X	**III. E.**

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GLOSSARY AND ABBREVIATIONS

ADI	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 Microorganism
GMO	Genetically Modified Organism
ORF	Open Reading Frame
PCR	Polymerase Chain Reaction
PMEM	Post-Market Environmental Monitoring
PMM	Post-Market Monitoring
QPS	Qualified Presumption of Safety
RT-PCR	Reverse-transcription PCR
SCF	Scientific Committee on Foods
TUL	Tolerable Upper Intake Level
VBNC	Viable But Non-Cultivable

Adjuvant: A substance that, when co-administered with an antigen, increases 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 antiparasitic agents.

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 multiplication or of transferring genetic material 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.

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: Characteristic of the GMM or its product capable of causing potential adverse effects (Chapter II, page 6).

Hazard identification: The identification of characteristics of the GMM or its product capable of causing potential adverse effects, 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 (Chapter II, page 6).

Intended effects are those changes that are targeted to occur due to the genetic modification, and that fulfil the objectives of the genetic modification (see Chapter II).

Microorganisms: Any microbiological entity, cellular or non-cellular, capable of multiplication 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 include filamentous fungi, yeasts, protozoa and microalgae.

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

Parental organism/strain: The microorganism with direct genealogical link to the GMM.

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.

Recipient organism/strain: The microorganism that is subjected to modifications leading to the desired outcome. The recipient organism/strain gives rise to the GMM.

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 are changes in the GMM resulting from its genetic modification other than the intended effects (see Chapter II).

APPENDIX

CORRELATION TABLE COMPARING THE REQUIRED INFORMATION ACCORDING TO REGULATION (EC) 1829/2003 AND THIS GUIDANCE DOCUMENT (GD)

If the product contains or consists of GMMs, specific information has to be included as stipulated under Art. 5 of Regulation (EC) 1829/2003 (no shading) referring to annexes II, III, and VII of Directive 2001/18/EC (grey shading). For feed (Art. 17) the same correlation system is valid.

	Text Regulation or Directive	GD Chapter/Section
Regulation (EC) No 1829/2003		
Art. 5(3)		
(a)	the name and the address of the applicant;	III. A.
(b)	the designation of the food, and its specification, including the transformation event(s) used;	III. A.
(c)	when applicable, the information to be provided for the purpose of complying with Annex II to the Cartagena Protocol on Biosafety to the Convention on Biological Diversity (hereinafter referred to as the Cartagena Protocol);	III. A.
(d)	when applicable, a detailed description of the method of production and manufacturing;	III. B.2.1. - III. B.2.2.
(e)	a copy of the studies, including, where available, independent, peer-reviewed studies, which have been carried out and any other material which is available to demonstrate that the food complies with the criteria referred to in Article 4(1);	III. B. in general
(f)	either an analysis, supported by appropriate information and data, showing that the characteristics of the food are not different from those of its conventional counterpart, having regard to the accepted limits of natural variations for such characteristics and to the criteria specified in Article 13(2)(a), or a proposal for labelling the food in accordance with Article 13(2)(a) and (3);	III. B.2.3. - III. B.2.4.
(g)	either a reasoned statement that the food does not give rise to ethical or religious concerns, or a proposal for labelling it in accordance with Article 13(2)(b);	Not in the scope of this Guidance
(h)	when appropriate, the conditions for placing on the market the food or foods produced from it, including specific conditions for use and handling;	III. A.
(i)	methods for detection, sampling (including references to existing official or standardised sampling methods) and identification of the transformation event and, when applicable, for the detection and identification of the transformation event in the food and/or in foods produced from it;	III. B.1.4.5.
(j)	samples of the food and their control samples, and	Not in the scope of this

	Text Regulation or Directive	GD Chapter/Section
	information as to the place where the reference material can be accessed;	Guidance
(k)	when appropriate, a proposal for post-market monitoring regarding use of the food for human consumption;	III. D.
(l)	a summary of the dossier in a standardised form;	Not in the scope of this Guidance
Directive 2001/18/EC		
Annex II/D.1	Conclusions of the potential environmental impact from the release or the placing on the market of GMOs	III. C.2.
Annex III/A		
	I. GENERAL INFORMATION	
A	Name and address of the notifier (company or institute)	III. A.
B	Name, qualifications and experience of the responsible scientist(s)	III. A.
C	Title of the project	III. A.
	II. INFORMATION RELATING TO THE GMO	
A	**Characteristics of (a) the donor, (b) the recipient or (c) (when appropriate) parental organism(s)**	
	1. scientific name	III. B.1.1.1., III. B.1.2.1.
	2. taxonomy	III. B.1.1.1., III. B.1.2.1.
	3. other names	III. B.1.1.1., III. B.1.2.1.
	4. phenotypic and genetic markers	III. B.1.1.2.
	5. degree of relatedness between donor and recipient or between parental organisms	III. B.1.1.3.
	6. description of identification and detection techniques	III. B.1.1.4.
	7. sensitivity, reliability (in quantitative terms) and specificity of detection and identification techniques	III. B.1.1.4.
	8. description of the geographic distribution and of the habitat of the organism including information on natural predators, preys, parasites and competitors, symbionts and hosts	III. B.1.1.5., III. B.1.2.1.
	9. organisms with which transfer of genetic material is known to occur under natural conditions	III. B.1.1.6.
	10. verification of the genetic stability of the organisms and factors affecting it	III. B.1.1.7.

	Text Regulation or Directive	GD Chapter/Section
	11. pathological, ecological and physiological traits	III. B.1.1.8., III. B.1.2.1.
	12. nature of indigenous vectors	III. B.1.1.6.
	13. history of previous genetic modifications	III. B.1.1.10.
B	**Characteristics of the vector**	
	1. nature and source of the vector	III. B.1.3.1.
	2. sequence of transposons, vectors and other non-coding genetic segments used to construct the GMO and to make the introduced vector and insert function in the GMO	III. B.1.3.1.
	3. frequency of mobilisation of inserted vector and/or genetic transfer capabilities and methods of determination	III. B.1.3.2.
	4. information on the degree to which the vector is limited to the DNA required to perform the intended function	III. B.1.3.2.
C	**Characteristics of the modified organism**	
	1. Information relating to the genetic modification	III. B.1.3.
	(a) methods used for the genetic modification	III. B.1.3.2.
	(b) methods used to construct and introduce the insert(s) into the recipient or to delete a sequence(s)	III. B.1.3.2.
	(c) description of the insert and/or vector construction	III. B.1.3.2.
	(d) purity of the insert from any unknown sequence and information on the degree to which the inserted sequence is limited to the DNA required to perform the intended function	III. B.1.3.2.
	(e) methods and criteria used for selection	III. B.1.3.2.
	(f) sequence, functional identity and location of the altered/inserted/deleted nucleic acid segment(s) in question with particular reference to any known harmful sequence	III. B.1.3.2.
	2. Information on the final GMO	III. B.1.4.
	(a) Description of the genetic trait(s) or phenotypic characteristics and in particular any new trait and characteristics which may be expressed or no longer expressed	III. B.1.4.1.
	(b) Structure and amount of any vector and/or donor nucleic acid remaining in the final construction of the modified organism	III. B.1.4.2.

	Text Regulation or Directive	GD Chapter/Section
	(c) Stability of the organism in terms of genetic traits	III. B.1.4.3.
	(d) Rate and level of expression of the new genetic material. Method and sensitivity of measurement	III. B.1.4.4.
	(e) Activity of the expressed protein(s)	III. B.1.4.4.
	(f) Description of identification and detection techniques including techniques for the identification and detection of the inserted sequence and vector	III. B.1.4.5.
	(g) Sensitivity, reliability and specificity of detection and identification techniques	III. B.1.4.5.
	(h) History of previous releases or uses of the GMO	III. B.1.4.7.
	(i) Considerations for human health and animal health as well as plant health	III. B.1.4.8. III. B.2.4.
	III. INFORMATION RELATING TO THE CONDITIONS OF RELEASE AND THE RECEIVING ENVIRONMENT	III. B.4.2. - III. B.4.3.
	IV. INFORMATION RELATING TO THE INTERACTIONS BETWEEN THE GMOs AND THE ENVIRONMENT	III. B.4.2. - III. B.4.3.
Annex VII	MONITORING PLAN	III. E.