GUIDANCE DOCUMENT OF THE SCIENTIFIC PANEL ON GENETICALLY MODIFIED ORGANISMS FOR THE RISK ASSESSMENT OF GENETICALLY MODIFIED MICROORGANISMS AND THEIR DERIVED PRODUCTS INTENDED FOR FOOD AND FEED USE

Adopted on 17 May 2006

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European Food Safety Authority

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About EFSA

The European Food Safety Authority (EFSA) was established and funded by the European Community as an independent agency in 2002 following a series of food scares that caused the European public to voice concerns about food safety and the ability of regulatory authorities to fully protect consumers.

In close collaboration with national authorities and in open consultation with its stakeholders, EFSA provides objective scientific advice on all matters with a direct or indirect impact on food and feed safety, including animal health and welfare and plant protection. EFSA is also consulted on nutrition in relation to Community legislation.
EFSA's work falls into two areas: risk assessment and risk communication. In particular, EFSA's risk assessments provide risk managers (EU institutions with political accountability, *i.e.* the European Commission, European Parliament and Council) with a sound scientific basis for defining policy-driven legislative or regulatory measures required to ensure a high level of consumer protection with regard to food and feed safety.

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Summary

The Scientific Panel on Genetically Modified Organisms (GMO Panel) adopted its guidance document for the risk assessment of genetically modified microorganisms (GMMs) and their derived products intended for food and feed use on 17 May 2006. The European Food Safety Authority (EFSA) and the GMO Panel have published the guidance on the EFSA web site for public consultation prior to the final adoption of this document.

This document provides guidance for the scientific risk assessment of genetically modified microorganisms (GMMs) and their derived products intended for food and feed use. In particular, it provides detailed guidance to assist in the preparation and presentation of applications to market GMMs and their products for food and/or feed use, according to Regulation (EC) 1829/2003 (EC, 2003a). In addition, this document provides guidance for the risk assessment of food and feed produced using GMMs, irrespective of whether they fall in the scope of Regulation (EC) 1829/2003 or not. Issues related to risk management of GMOs (traceability, labelling) are outside the scope of the guidance document.

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

Guidance for the presentation of applications can be found in the Annexes to the guidance document. These include details on the key component parts of the application, on the format of technical dossiers and on the summary of applications. There are also specifications on the submission of samples of GM microorganisms and derived product to DG Joint Research Centre.

Key words: GMOs, GM microorganisms, GM food, GM feed, guidance, applications, Regulation (EC) 1829/2003, Directive 2001/18/EC, food safety, feed safety, environment.

Table of Contents

Foreword

Genetic modification, genetic engineering or recombinant-DNA technology, first applied in the 1970's, is one of the newest methods to introduce novel traits to microorganisms, plants and animals. Unlike other methods, the application of this technology is strictly regulated. Before any genetically modified organism (GMO) or derived product can be placed on the EU market, it has to pass an approval system in which the safety for humans, animals and the environment is thoroughly assessed. In line with the provisions of Regulation (EC) 1829/2003 on genetically modified food and feed, which applies from April 18, 2004, the Commission has asked the European Food Safety Authority (EFSA) to publish detailed guidance to assist the applicant in the preparation and presentation of the application for the authorisation of genetically modified (GM) food and/or feed. A first guidance document for the risk assessment of genetically modified plants and derived food and feed has already been published by EFSA (EFSA, 2004b).

The present document provides detailed guidance for the assessment of genetically modified microorganisms (GM microorganisms) and their derived products intended for food and feed use. This guidance complements, but does not replace, other requirements, as set out in specific legislation, that a product has to fulfil in order to be approved for the European market.

This document was compiled by the Scientific Panel on Genetically Modified Organisms (GMO Panel) of EFSA, consisting of the following members:
Christer Andersson, Detlef Bartsch, Hans-Joerg Buhk, Howard Davies, Marc De Loose, Michael Gasson, Niels Hendriksen, John Heritage, Sirpa Kärenlampi, Ilona Kryspin-Sørensen, Harry Kuiper, Marco Nuti, Fergal O'Gara, Pere Puigdomenech, George Sakellaris, Joachim Schiemann, Willem Seinen, Angela Sessitsch, Jeremy Sweet, Jan Dick van Elsas and Jean-Michel Wal.

The following *ad hoc* experts also contributed:

Bevan Moseley, Ingolf F. Nes and Paul Ross.

The draft document was published on the EFSA website in July 2005 for a two and a half month period of public consultation. The GMO Panel considered all comments relating to the risk assessment of GMOs before preparing its revised guidance document. The GMO Panel did not consider issues related to risk management of GMOs (traceability, labelling). Political and socio-economic issues are also outside the remit of the Panel. The guidance document was adopted by the GMO Panel on 17 May 2006. The GMO Panel will regularly review this guidance in the light of experience gained, technological progress and scientific developments. By establishing a harmonised framework for risk assessment, this document should provide useful guidance both for applicants and risk assessors.

Terms of reference

In accordance with Articles 5(8) and 17(8) of the Regulation (EC) 1829/2003 (EC, 2003a) on genetically modified food and feed, in a letter dated 27 October 2003, the European Commission has requested the European Food Safety Authority (EFSA), to publish detailed guidance to assist applicants[2] in the preparation and presentation of applications for the authorisation of GM food and/or feed (ref. SANCO/D4/KM/cw/D/440551(2003)).

A guidance document for the risk assessment of GM plants and derived food and feed has already been published by EFSA (EFSA, 2004b).

In addition, the Commission requested EFSA, in a letter dated 1 February 2005, to provide guidance on the scientific information necessary for the risk assessment for food and feed produced using GMMs, irrespective of whether they fall in the scope of Regulation (EC) 1829/2003 or not (ref. SANCO/D4/KN/cw/D/440010 (2005)). The guidance should cover both food/feed and food/feed ingredients produced using GMMs as well as substances such as additives, vitamins and flavourings produced by GMMs.

Mandate of EFSA and the GMO Panel

Consistent with Regulation (EC) 178/2002 (EC, 2002c), EFSA is mandated to provide scientific advice and scientific technical support for the Community's legislation and policies in all fields that have a direct or indirect impact on food and feed safety. EFSA is required to provide independent information on all matters within these fields and communicate on risks. EFSA shall contribute to a high level of protection of human life and health. It shall take account of animal health and welfare and also plant health and the environment. This responsibility is placed in the context of the operation of the internal market.

The Scientific Panel on Genetically Modified Organisms, hereafter referred to as the GMO Panel, deals with questions on GMOs as defined in Directive 2001/18/EC (EC, 2001a), including plants, microorganisms and animals, relating to their deliberate release into the environment and their use in genetically modified food and feed including their derived products (EC, 2001a; EC, 2003a; EFSA, 2002).

2 - The term "applicant" is used hereafter as a generic reference to the official body submitting the application.

I. INTRODUCTION

1. Scope of the document

This document provides guidance for the scientific risk assessment of genetically modified microorganisms (GMMs)[3] and their derived products intended for food and feed use. In particular, it provides detailed guidance to assist in the preparation and presentation of applications to market GMMs and their products for food and/or feed use, according to Articles 5(8) and 17(8) of Regulation (EC) 1829/2003 (EC, 2003a). In addition, this document provides guidance for the risk assessment of food and feed produced using GMMs, irrespective of whether they fall in the scope of Regulation (EC) 1829/2003 or not.

Not all requirements of the guidance document may be applicable for all products.

For the purpose of this guidance document, the types of genetically modified microorganisms (GMMs) covered include both prokaryotes and eukaryotes[4]. This document does not cover the use of tissue cultures of plant or animal cells[5], nor does it cover issues related to risk management (traceability, labelling, etc.). Socioeconomic and ethical issues are also outside the scope of this guidance. This guidance does not cover the contained use of GMMs (Directive 90/219 EEC; EC, 1990, Directive 98/81/EC; EC, 1998), nor does the guidance cover the deliberate release into the environment of GMMs for any other purpose than for the 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. A separate guidance document has been produced for the risk assessment of genetically modified plants and derived food and feed (EFSA, 2004b).

This document provides guidance on:

1) the drawing up of Annex IIIA of the Directive 2001/18/EC (EC, 2001a) on the deliberate release into the environment of genetically modified organisms (GMOs),

2) the preparation of an environmental risk assessment as stated in Annex II paragraph D.1, and

3) the establishment of an environmental monitoring plan according to Annex VII of that Directive.

This guidance is without prejudice to the supplementary guidance notes 2002/623/EC (EC, 2002a) and 2002/811/EC (EC, 2002b) established within the framework of Directive 2001/18/EC.

The document addresses the requirements of Regulation (EC) 1829/2003 and is structured essentially according to the requirements set out in Articles 5(5) and 17(5) of the Regulation (EC) 1829/2003, *i.e.* taking into account Annexes IIIA, IID1 and VII of Directive 2001/18/EC. This guidance also takes into account all relevant parts of the Directives 90/219 EEC and 98/81/EC on the contained use of GMMs (EC, 1990; EC, 1998).

3 - Genetically modified organisms are defined in Directive 2001/18 (EC) (EC, 2001a) as organisms in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination.
4 - Prokaryotic microorganisms include archaea and eubacteria. Eukaryotic microorganisms include yeasts, filamentous fungi, protozoa and microalgae (Heritage et al., 1996).
5 - Directive 98/81/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".

Food additives (Directive 89/107/EEC; EC, 1989), flavourings (Directive 88/388/EEC; EC, 1988) and feed additives (Regulation (EC) No 1831/2003; EC, 2003b) and certain products used in animal nutrition (Directive 82/471/EEC; EC, 1982) containing, consisting of, or produced from GMMs, fall under Regulation 1829/2003 and therefore also fall within the scope of this guidance document.
As regards the use of GMMs as plant protection products, bioremediation agents, biofertilisers or phytostimulators, these applications will fall into the wider scope of the Directive 2001/18/EC, and further guidance in this area will be developed. Although this document focuses on GMMs and derived food and feed, the principles of risk assessment of GMMs intended for other applications when products are likely to enter the food or feed chains, is unlikely to differ significantly with respect to their presence in food or feed.

In general, a risk assessment of the GMM includes the nature of the genetic modification and the presence of the GMM and its derivatives, including DNA, in the final food or feed product. GMMs used for food and feed purpose can be differentiated on the basis of their use in i) GMMs deliberately released into the environment, according to Directive 2001/18/EC, and used as food or feed or contained in food or feed; ii) GMMs deliberately released into the environment, according to Directive 2001/18/EC, and used for the production of food or feed; iii) GMMs used for the production of food or feed under 'contained use' according to conditions defined in Directive 90/219/EEC (EC, 1990).

For uses as in i) and ii), a full risk assessment according to Regulation (EC) 1829/2003 in combination with Directive 2001/18/EC is required and is covered by this guidance. With regard to uses as in iii), *i.e.* GMMs used for food or feed production under containment, this guidance covers the assessment of the final product to be used as food or feed for the placing in 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 90/219/EEC.

In cases of GM food or feed produced under containment the applicant should submit not only the information relevant to Regulation (EC) 1829/2003 but should also make available the risk assessment undertaken in compliance with Directive 90/219/EEC and the implemented national legislation, thereby covering the assessment of the GMM itself and taking account of the genetic modification and the gene products derived therefrom. There may be circumstances in which the DNA as such introduced into a GMM gives cause for concern and in this case it needs to be subjected to risk assessment. Data on the absence of DNA need to be very robust in such instances. Indeed, given that no method will give absolute proof that DNA is absent, there is a case to undertake a specific safety assessment based on the minimal level of DNA that might be detected.

II. THE RISK ASSESSMENT STRATEGY

The risk assessment strategy is the driving force and justification for the information requirements.

1. Risk assessment

Risk assessment is "a process of evaluation including the identification of the attendant uncertainties, of the likelihood and severity of an adverse effect(s)/event(s) occurring to humans or the environment following exposure under defined conditions to a risk source(s)" (EC, 2000a). A risk assessment comprises hazard identification, hazard characterisation, exposure assessment and risk characterisation. A hazard is the potential of an identified source to cause an adverse effect.

The sequential steps in risk assessment of GMOs identify characteristics that may cause adverse effects, evaluate their potential consequences, assess the likelihood of occurrence and estimate the risk posed by each identified characteristic of the GMOs.

1.1. 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.

1.2. Hazard characterisation

Hazard characterisation involves an assessment of the consequences of exposure to a hazard. It involves the qualitative or, whenever possible, quantitative description of the nature of the hazard and their respective attendant uncertainties. It may also be described as determining the potential severity of adverse effects following exposure to a hazard.

1.3. Exposure assessment

Exposure assessment determines the probability and the likely levels of exposure in the human population.

1.4. Risk characterisation

Risk characterisation is the qualitative or, whenever possible, quantitative estimate of the probability of occurrence and severity of adverse effect(s) or event(s) in a given population under defined conditions based on hazard identification, hazard characterisation and exposure assessment (SSC, 2000), including the attendant uncertainties. Chapter IV describes how this step should be carried out and gives examples of issues to be addressed.

"Qualified Presumption of Safety" (QPS)

In a recent Opinion (EFSA, 2005), the Scientific Committee of EFSA took steps towards the establishment of a generic approach to the safety assessment by EFSA of microorganisms used in food and feed and the production of food or feed additives.

This proposes the introduction of the concept of the "Qualified Presumption of Safety" (QPS), which is intended to be applied to selected groups of microorganisms. This opinion specifically excludes microorganisms developed using recombinant DNA technology for strain improvement, since these are covered by separate existing legislation (Regulation (EC) 1829/2003). The EFSA Scientific Colloquium on QPS (EFSA, 2004c) addressed the status of GMMs, with particular reference to self-cloning. It was concluded that in such cases, there appears to be no scientific basis for the exclusion of self-cloned GMMs from a QPS risk assessment in the future. A list of QPS organisms is being established and will increase in time.

2. Risk assessment of the GMMs and derived products for human and animal health

GMMs and their products intended for human and animal consumption form a broad spectrum ranging from a single compound used in food or feed at one end to pure cultures of viable GMMs at the other end. Amino acids or vitamins that have been purified by crystallisation would represent examples at one end of this spectrum and cultures of probiotic microorganisms or dairy starters at the other extreme. In the middle of the spectrum lie both products of genetically modified microorganisms, such as dairy products, in which the viable GMMs persist and products in which it is not expected the presence of viable GMMs but where traces of the transgenic event may persist, for example crude enzyme preparations produced by the lysis of microbial cells. Three groups of GMMs or derived food and feed may be distinguished:

Group 1: Single compounds or defined mixtures of compounds derived from GMMs (*e.g.* amino acids, vitamins, pure enzymes);

Group 2: Complex products derived from GMMs but not containing viable GMMs nor unit length of any cloned (foreign) open reading frames (*e.g.* lysed cell extracts, some feed enzymes, wine, some beers, etc.);

Group 3: GMMs and products containing viable GMMs or genetically intact cloned (foreign) DNA (*e.g.* live or heat killed starter cultures and probiotic cultures, some beers, cheeses, yoghurts, etc.).

Foods and feeds consisting of or containing single compounds or defined mixtures obtained from a GMM require a different assessment from foods and feeds containing either viable or non-viable GMMs. The level of scrutiny and the focus of the assessment will also differ for food and feed consisting of or containing single compounds or defined mixtures of chemically purified and defined compounds derived from GMMs compared with other food and feed produced using GMMs in which no purification process has been carried out but which do not contain viable GMM cells. The most intense scrutiny is reserved for products containing viable GMMs, whether as a component of a food or feed or as a pure culture used, for example, as a probiotic or as starter culture in the food industry (Table 1). Only limited information focusing on the production system is required to perform a risk assessment on single compounds. When GMMs are not recoverable from a product but where purification of the product is limited, information required for risk assessment will be more extensive than for single products. It will be necessary to understand the processes by which the GMM

6 - *Self-cloning, as defined by Directive 98/81/EC (EC, 1998), consists in the removal of nucleic acid sequences from a cell of an organism which may or may not be followed by reinsertion of all or part of that nucleic acid (or a synthetic equivalent) with or without prior enzymic or mechanical steps, into cells of the same species or into cells of phylogenetically closely related species which can exchange genetic material by natural physiological processes where the resulting microorganism is unlikely to cause disease to humans, animals or plants.*

has been inactivated in the product and the degree to which traces of the transgenic event may be detected in the product. When live GMMs persist in a product, the most extensive information will be required to permit a scientific risk assessment.

In the case of food or feed consisting of or containing GMMs obtained by self-cloning[6], applicants should address all of the requirements needed for the risk assessment of GMMs and derived food or feed as described in this document. A restricted information set might be sufficient for risk assessment when food and feed are derived from self-cloned GMMs but not containing viable GMMs. In such cases, however, the assessment should be performed on a case-by-case basis. In cases in which self-cloning has been performed using different strains of the same or closely related species, information on the history of use and on the safety of the species should be provided. Species that are recognised to have strains that are pathogenic should be evaluated for this trait.

The level of scrutiny of the risk assessment depends on the history of use of the recipient and donor strains (depending on the sequences to be cloned) as well as of the modification itself. The risk assessment of GMMs will be simplified when the qualified presumption of safety (QPS) of microorganisms in the food and feed chains has been introduced. In particular, the risk assessment will need only to focus on relevant information not available in the QPS qualification in cases when the parental or recipient and the donor strains have been granted the status of QPS or if they belong to a taxonomic group with QPS status for the same end-use.

3. Comparative approach

The risk assessment strategy for GMMs seeks to deploy appropriate methods and approaches to focus not only on intended modifications, but also on the potential unintended (unexpected) outcomes of the genetic modification process itself. The strategy adopted in this guidance document is based on comparison of the GMM or GM food or feed with its conventional counterpart. The comparative approach is based on the concept that a conventional counterpart with a history of safe use can serve as a baseline for the environmental and food and feed risk assessment of a particular GMM. For this, the concepts of “familiarity” and “substantial equivalence” were developed by the OECD (OECD, 1993a & OECD, 1993b) and further elaborated by ILSI (ILSI, 1999) and WHO/FAO (WHO/FAO, 2001b). The purpose of the risk assessment is to identify new or altered hazards relative to the conventional counterpart. The comparison should be considered as the first step of the risk assessment. In the second step, the environmental and food or feed safety or nutritional impact of the identified differences, whether intended or unintended, should be assessed.

Concepts of “familiarity” and “body of knowledge”

The concept of “familiarity” refers to the fact that most GMMs to be used for food or feed purposes belong to well-characterised microbial species. This “familiarity” allows the risk assessor to draw on previous knowledge and experience with the introduction of similar microorganisms into food and the environment. “Familiarity” will also derive from the knowledge and experience available from the risk/safety analysis conducted prior to the scale-up of the microorganism in a particular environment (OECD, 1993a). The concept of “history of safe use” was described in detail by ILSI (ILSI, 1999) and was discussed further at the EFSA Scientific Colloquium on QPS (EFSA, 2004c), when the term “body of knowledge” was proposed as a replacement for “familiarity”. Neither of these concepts as such represents a reasonable certainty of no harm. It is the nature

and content of the body of knowledge that may or may not lead to such a conclusion. If the parental microorganism has been granted the proposed status of QPS for the same production conditions and final use as it is intended in the application, all the information on the history of safe use has already been assessed.

Concept of substantial equivalence

The concept of "substantial equivalence" is based on the rationale that an existing microorganism with a history of safe use as food or feed can serve as a comparator when assessing the safety of GM food and feed (OECD, 1993b). Application of this concept, also referred to as comparative risk assessment (Kok and Kuiper, 2003), serves to identify similarities and, in particular, differences between the GMM or derived food or feed and its conventional counterpart. The differences should then be assessed for their toxicological and/or nutritional impact on humans and animals. In some cases, a GM strain that has already been through a risk assessment and been approved for marketing in the EU could serve as the comparator if it has been shown to have a good safety record.

The application of the concept of substantial equivalence is not a risk assessment per se, but it structures the risk assessment process. The first step in the risk assessment is thus the comparative analysis of the molecular characteristics of the microorganism including, when relevant, its metabolic products. The comparisons should be made between microorganisms grown or used under the same conditions, if possible. The outcome of the comparative analysis will give further guidance to the second part of the risk assessment procedure, which may include specific toxicological and, when relevant, nutritional testing. The outcome should be the comparative safety of the GM food or feed and the traditional counterpart. When no appropriate comparator can be identified, a more straightforward risk and nutritional assessment of the GM food or feed should be carried out. This would be the case, for instance, when a trait or traits are introduced into a microorganism with the intention of significantly modifying the composition of the food or feed.

Intended and unintended effects

Intended effects are those that are targeted to occur due to the introduction or inactivation of gene(s) or DNA sequences, and that fulfil the objectives of the genetic modification. Intended alterations in the composition of a GMM compared with the parent may be identified by measurements of single compounds like newly expressed proteins, and the intended impact on metabolic flux (a targeted approach).

Unintended effects are consistent phenotypical differences between the GMM and its otherwise isogenic comparator that goes beyond the primary expected effect(s) of introducing or inactivating the target gene(s). Unintended effects may be predicted or explained in terms of current knowledge of microbiology and of the integration of metabolic pathways. Unintended effect(s) could also be due to genetic rearrangements. Insertion of new DNA sequences may lead to changes in the expression of particular genes in the recipient genome, metabolic perturbations and pleiotropic effects. It may also result in the synthesis of new fusion proteins. A starting point in the identification of potential unintended effects is the sequence analysis of regions flanking the insertion site to establish whether the insertion has occurred within, or in the proximity of, an endogenous gene. Sequence analysis should extend to identifying whether the introduced DNA interrupts a transcriptional unit, *e.g.* a polycistronic operon as well as whether it causes the synthesis of a fusion protein. In addition, pulsed field gel electrophoresis (PFGE) could be employed to generate restricted genomic DNA fingerprints to assess whether any gross genomic change has occurred. In microorganisms in which the genome sequence is available,

microarray technology and proteomics may be used to identify significant alterations in gene order and gene expression. A comparative and targeted analysis should be carried out of single compounds in the GMM and its conventional counterpart, which represent components of relevant metabolic and physiological pathways in the organism. If the GMM comprises a significant part of the diet, or leads to changes of intake of such GM food to certain sub-populations (children, the elderly, etc.) these components should include macronutrients, micronutrients and primary and secondary metabolites as well as known anti-nutrients but also whole GMMs (probiotics, starter cultures, etc.). The presence of known toxins, when relevant, should be analysed. Statistically significant differences between the GMM and its comparator that are not due to the intended modification may indicate the occurrence of unintended effects. These should be assessed specifically with respect to their safety and, when relevant, nutritional impact.

Considering the high level of gene mobility and the plasticity of microbial genomes, particular attention should be paid to the evaluation of differences in gene expression between the GMM and its conventional counterpart. This is particularly important when the genetic modification of the GMM is located on a multi-copy plasmid. In addition, the presence of naturally occurring changes or rearrangements within the genome of closely related strains in natural microbial populations should be considered as this provides a baseline of natural changes. Thus, scientific evidences should be provided in order to attribute the identified differences to the genetic modification event.

4. Environmental risk assessment and monitoring

The risk of adverse effects on the environment caused by a GMM depends on whether the GMM has access to and can survive in the natural environment. Therefore, an assessment of the ability of the GMM to survive and persist and spread in the environment is always needed. In this context, comparison with a conventional counterpart under the same conditions of use should be considered, when applicable. Further, the receiving environments for the GMM need to be identified. If material containing DNA from the GMM may gain access to the open environment, the possibility of gene transfer and selection of the transgene sequences should be assessed and the consequences evaluated.

For GMMs that have the potential to survive, persist and spread in the environment to which they may gain access it is necessary to identify and assess effects linked to the genetic modification that may result in adverse effects in any receiving environment on a case-by-case basis. The following points should be addressed when appropriate:

- the potential for survival and persistence in the receiving environment and any selective advantage that may be offered: in the case of selective advantage, its nature should be identified along with any potential for negative effects;
- the potential for gene transfer;
- the potential for negative effects or consequences based on interactions with indigenous microorganisms;
- possible effects on humans, animals and plants;
- possible effects or (non-reversible) perturbations on biogeochemical processes.

These points may be assessed by a combination of laboratory studies, micro- and mesocosm experiments and small-scale field releases to identify hazards and to quantify actual levels of exposure. However, based on the nature of the microorganisms in

question, a case-by-case approach should be followed. For example, for many starter and intestinal and/or probiotic organisms it could be envisaged that an exhaustive environmental risk assessment may not be relevant, given that these microorganisms may not be expected to survive or persist in external environments and in many cases would have limited direct contact with the environment. If, however, the genetic modification makes survival and persistence more likely, then a more extensive environmental risk assessment must be undertaken.

It is recognised that an environmental risk assessment is only as good as the state of scientific knowledge at the time it is conducted. Under current EU legislation, environmental risk assessment is required to identify uncertainties or risks beyond current knowledge and the limited scope of the environmental risk assessment. These include specific factors such as the impact of large-scale exposure of different environments, of exposure over long periods and cumulative long-term effects. Legislation requires that plans for monitoring for such effects are presented in the application.

The scientific knowledge and experiences gained from monitoring will in turn inform the risk assessment process. Thus, the results of monitoring provide opportunities to update the risk assessment continually in the light of any new knowledge.

5. The framework for risk assessment of genetically modified microorganisms and derived food and feed

The risk assessment of a GMM or a food or feed derived from a GMM consists of a step-by-step process that addresses different requirements described in Chapter III and summarized in Table 1 of this guidance document.

6. General recommendations

Whenever possible, applicants are encouraged to develop those GMMs in which only DNA essential to the modification of the trait in question is transferred to the microorganism for commercial release (ACRE, 2002; SSC, 2003b).

The choice of a particular marker gene should be given careful consideration. Particular attention should be given to the use of marker genes (EFSA, 2004a) that confer resistance to therapeutically relevant groups of antibiotics and, whenever possible, such markers should be avoided altogether.

At an early stage in the development of a GMM, some strain improvement considerations and strategies analogous to those suggested for genetically modified crops (ACRE, 2001) are relevant. Adoption of these strategies could help reduce potential risks and may avoid some unidentified risks in the environment. The overall aim is to reduce environmental exposure and the potential risks associated with transgenes and their products. Three principle approaches can be considered useful to achieve this:

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

7 - http://www.entransfood.com/RTDprojects/GMOCARE.
8 - http://www.food.gov.uk/science/research/researchinfo/foodcomponentsresearch/novelfoodsresearch/g02programme/

7. Forthcoming developments

To increase the chances of detecting the potential for unintended effects due to the genetic modification of organisms, profiling technologies such as transcriptomics, proteomics and metabolomics, extend the breadth of comparative analyses (Kuiper *et al.*, 2003; ILSI, 2004). The utility and applicability of these technologies in the detection of altered gene and protein expression and metabolite composition in GM crops and their derived foods has been under scrutiny in specific research projects funded, for example, by EU FP5 (GMOCARE project[7]) and the UK Food Standards Agency (G02 research programme[8]). These technologies may also be helpful in the detection of intended and unintended effects in GMMs. Since many complete genome sequences are already available in databases, these tools may be more easily applied to microorganisms than they are currently to crop plants. The applicability of metabolomic techniques, such as gas chromatography coupled to mass spectrometry (GC-MS) and off-line liquid chromatography (HPLC) coupled to nuclear magnetic resonance (NMR), for the simultaneous analysis of a wide variety of metabolites in GMOs and their conventional counterparts has been demonstrated. These non-targeted approaches may be of particular relevance for GMMs with specific metabolic pathways modified, *e.g.* those leading to enhanced nutritional profiles, obtained through the insertion of single or multiple genes.

Further exploration of profiling approaches is needed with respect to the evaluation of specificity, sensitivity and reproducibility. Profiling methods are not aimed at replacing conventional analyses but may be useful to confirm and complete other data. It must be appreciated however that many "omic" profiling technologies are not yet fully developed; since they are interfaced with the physiological status of cells, this may limit their applicability to certain GMMs. Thus, application of these tools is not a pre-requisite for the risk assessment of GMMs.
Nevertheless, the development of appropriate robust profiling technologies with particular emphasis on achieving harmonised and validated conditions for application together with the availability of appropriate functional databases for comparative analysis is strongly recommended.

8. Regulatory background for the risk assessment of GMOs, GM food and GM feed at Community level

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

In cases in which a GMM is used as the source of a product, the applicant should follow the specific legislation and the corresponding guidelines, if available, when preparing an application to market that product. To facilitate the assessment of the genetic modification, the applicant should follow the relevant parts of the present guidance document.

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

Regulation (EC) 178/2002 (EC, 2002c) lays down the general principles and requirements of food law, procedures in food safety and establishes the European Food Safety Authority (EFSA) and its tasks.

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

According to Regulation (EC) 1829/2003 (EC, 2003a), GM food and feed may only be authorised for placing on the market after a scientific assessment of any risks that they might present for human and animal health and, as the case may be, for the environment.

An application should be accompanied by the particulars specified by Articles 5(3) and (5) and/or Article 17(3) and (5) of the Regulation for GM food and feed, respectively. The European Commission has established implementing rules for the application of these Articles, including rules concerning the preparation and the presentation of the application (Regulation (EC) 641/2004; EC, 2004b).
EFSA uses the GMO EFSA-net to make the application available to the Member States and the Commission and makes the summary of the application available to the public.

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

The principles regulating the deliberate release of GMOs into the environment are laid down in Directive 2001/18/EC (EC, 2001a). Part C of the Directive deals with placing on the market of GMOs as, or in, products.

Annex IIIA of the Directive details the required information on which to base the risk assessment for organisms other than higher plants, *e.g.* GMMs. The principles for the environmental risk assessment, including aspects of human and animal health, are laid down in Annex II of the Directive. Several supporting documents have been prepared to assist the applicant. Commission Decision 2002/623/EC (EC, 2002a) establishes guidance notes on the objective, elements, general principles and methodology of the environmental risk assessment referred to in Annex II to Directive 2001/18/EC. Council Decision 2002/811/EC (EC, 2002b) establishes guidance notes supplementing Annex VII to the Directive, describing the objectives and general principles to be followed to design the environmental monitoring plan. The Directive also introduces an obligation to propose a monitoring plan in order to identify and trace any direct or indirect, immediate, delayed or unforeseen effects on human health or the environment of GMOs as, or in, products after they have been placed on the market.
Council Decision 2002/812/EC (EC, 2002e) establishes the summary notification information format (SNIF).

Contained use of genetically modified microorganisms (Directive 98/81/EC)

The contained use of genetically modified microorganisms is regulated by Directive 90/219/EEC (EC, 1990), as amended by Directive 98/81/EC (EC, 1998).

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

Placing on the market of feed additives is authorised under Regulation (EC) 1831/2003 on additives for use in animal nutrition (EC, 2003b). In addition, feed additives containing, consisting of, or produced from GMOs fall within the scope of Regulation (EC) 1829/2003.

III. INFORMATION REQUIRED IN APPLICATIONS FOR GM MICROORGANISMS (GMMs) AND/OR DERIVED PRODUCTS[9]

A. GENERAL INFORMATION

1. The name and address of the applicant (company or institute).

2. The name, qualification and experience of the responsible scientist(s) and contact details of the person responsible for all dealings with EFSA.

3. The title of the project.

4. The scope of the application, as defined in Annex II.

5. 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, etc.

6. Where applicable, a detailed description of the method of production and manufacturing.

7. 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. INFORMATION RELATING TO THE GMM

Information relating to the GMM should include the most recent taxonomic classification and should identify the specific characteristics of the organism (OECD, 2003). This will allow for species-specific analyses, *e.g.* the known occurrence in the genus/species of specific toxins that are typically expressed at low levels in the unmodified recipient strains, but that may be unintentionally increased following the genetic modification process. Information should be provided on all issues of potential concern, such as the presence of natural toxins, allergens or virulence factors. Data should be provided on the previous use of the recipient organism and, when synthetic sequences are used for the genetic modification event, of the donor organism(s).

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

The applicant should provide a comprehensive description of the recipient microorganism or the parental strain in the case of a microorganism in which the endogenous genetic material has been modified. Its history of safe use should be described. In cases in which microorganisms that contain virulence determinants are used as recipients or parental organisms, their use must be justified in the application. In case of a parental or recipient microorganism with the status of QPS for the equivalent end use, the information requirements will be reduced (see Table 1). Information relating to the recipient or (when appropriate) the parental organism must include the following:

9 - *Not all the points included will apply in each case. Where the provision of information on a particular item does not apply for a particular application, reasons must be given for the omission of such data from the dossier.*

1.1 Identity

This should include common name, strain designation, information about the source of the strain, accession number from a recognised culture collection, if available. In the case of a novel isolate or a strain that has not been extensively studied, any issues relating to its use in food or feed should be addressed by the tests carried out to confirm identity of the strain.

1.2 Taxonomy

The most detailed description possible should be provided and should include (a) genus, (b) species, (c) subspecies (if appropriate) and (d) strain. The most appropriate taxonomic classification of the organism should be provided. Methods used for the taxonomic identification, down to strain level, should also be provided. The use of the most recent molecular and phenotypic techniques, such as metabolic profiling, used to establish the identity of the organism is recommended. For fungi, it is important to indicate the teleomorph/anamorph (sexual or asexual) state.

1.3 Other names

When appropriate, the generic name, commercial name, previous name(s), etc. by which the GMM is known should be provided.

1.4 Phenotypic and genetic markers

Phenotypic and genotypic information relevant to identification, genetic stability and/or safety should be provided, not only for the recipient strain, but also for related microorganisms, if appropriate. This should include any information relating to pathogenicity, potential immunological impact and human and animal health and the environment, when appropriate.

1.5 Degree of relatedness between recipient and donor(s)

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

1.6 Description of identification and detection techniques

These should be described in detail. A genetic fingerprint of the recipient strain should be provided, to identify it unequivocally, and, if appropriate, to permit its detection and quantification in the environment. The use of the most recent and reliable molecular techniques and, if possible, more than one, is recommended.

1.7 Sensitivity, reliability and specificity of the detection techniques

The choice of detection and identification techniques should be justified and their sensitivity, reliability, specificity and validation, should be provided.

1.8 Source and natural habitat of the recipient microorganism

Information should be provided on the habitat(s) in which the microorganism is found naturally. The source should be specified, whether the recipient is a wild strain (occurring naturally in that habitat) or a strain provided by a recognised culture collection. The diversity of strains and potential for interactions between the recipient

and other organisms in the normal habitat should be considered. This is particularly relevant in cases in which the GMM that is the subject of an application will be deliberately released into the environment. The description of the habitat where the microorganism has been isolated can be particularly relevant when the microorganism comes from extreme environmental conditions (*e.g.* very high temperatures) and the consequences of its adaptation (*e.g.* changes in the metabolic activity) to a different habitat should be evaluated.

1.9 Organisms with which transfer of genetic material is known to occur under natural conditions

Information based on the available peer-reviewed literature is sufficient. When there is the possibility of natural transfer of genetic material to other organisms, the potential consequences for the intended release of the derived GMM should be evaluated. The OECD is currently preparing a consensus document on this topic.

1.10 Information on the genetic stability of the recipient microorganism

Factors affecting the genetic stability should be specified (*e.g.*, insertion sequences, transposons, integrons, plasmids, prophage). Taking into account the high level of mobility that is typical of microbial genomes, the absence of any negative effect on human and animal health related to the genetic mobility (instability) should be assessed.

1.11 Pathogenicity, ecological and physiological traits

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

a) classification of hazard according to the current Community legislation concerning the protection of human health and/or the environment, and specifying to which risk group the microorganism belongs (Directive 2000/54/EC; EC, 2000c);

b) information on the doubling time and on the mode of reproduction;

c) information on survival, including the ability to form spores or other survival structures;

d) pathogenicity: information relating to infectivity, toxigenicity, virulence, allergenicity should be provided, as appropriate. Considering that the presence of a particular virulence factor in microorganisms is very often a strain-dependent characteristic, the absence of any factor related to pathogenicity and human/animal health should be established for the specific recipient strain. Information on pathogenicity should not only be provided for the recipient strain, but also for related strains and species. Information on the ability to colonise other organisms should be provided. In particular, applicants should provide information on the viability and ability of the recipient microorganism to survive in the gastrointestinal tract of humans or animals consuming the derived GMM or its product. In addition, information regarding any probiotic or immunomodulatory properties, whether advantageous or disadvantageous, should be provided. The risk assessment should address the health aspect for the whole human population, including immunocompromised individuals, infants and the elderly;

e) antibiotic resistance: information is required relating to the presence of genes that confer antibiotic resistance, in particular those that confer resistance to antimicrobial agents used in human and/or animal therapy. Information should also be provided on their location within the genome and on their potential for transfer

to other organisms; detection of the presence of resistance determinants should be carried out using both phenotypic and genotypic methods. The techniques used should be justified. The use of at least one phenotypic technique associated with at least one molecular technique is strongly recommended. In particular, antibiotic resistances not normally associated with the GMM genus or species should be highlighted. Microorganisms in which antibiotic resistance is conferred by an inactivating mechanism encoded by a gene that is located on a mobile genetic element and targeting an agent(s) in clinical or veterinary use should not be used in the recipient. The level of gene expression and the potential for the induction of gene expression should be evaluated when antibiotic resistances of particular concern are observed;

f) involvement in environmental processes: any information relating to the involvement of the recipient or parental organism in degradation of organic compounds, nutrient turnover, etc., should be provided, when appropriate.

1.12 Information on indigenous mobile genetic elements

The presence of known indigenous mobile genetic elements such as plasmids, transposons, integrons, prophage, sex factors or other genetic elements that could increase the likelihood of the mobilisation of genetic material should be noted. Any possible information regarding the nature, sequence, frequency of mobilisation and presence of genes with implications for safety should be provided.

1.13 Description of its history of use

Information should be provided relating to the previous use or unintended presence (*e.g.* as a contaminant) in food or feed. Information on the history of use in food or feed should, whenever possible, be supported by scientific evidence and applicants should provide evidence of safe use, preferably under conditions as close as possible to those anticipated for the derived GMM. The history of use should include information on how the microorganism is typically cultivated, transported and stored and on its viability during the product shelf-life. When a history of safe use is available for other strains belonging to the same species or genus, relevant information should be provided. A history of safe use is not sufficient by itself for a risk assessment, but it may represent a reasonable likelihood of no harm. The history of safe use should be evaluated on a case-by-case basis. The whole human population, including vulnerable groups, should be considered. When no history of safe use is recognised, the recipient strain should be fully assessed for safety.

1.14 History of previous genetic modifications

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

2. Characteristics of the donor organism(s)

In addressing the requirements listed below it should be remembered that, in many cases, the most important information required for a risk assessment is the source and nature of the gene(s) to be inserted rather than the characteristics of the donor. Information should, however, be provided on the donor organism(s). If relevant, information on organisms related to the donor(s) should be provided, for example, if a strain related to the donor elaborates a toxin that is known to be absent from the donor organism. 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. Not all genetic modification requires a donor organism. Synthetic DNA sequences may be used to introduce novel gene(s) into organisms. In such cases, the rationale for the design and use of synthetic sequences must be described in full by the applicant. Alternatively, the genome of the recipient organism may be modified in such a way that does not employ foreign DNA – so called self-cloning. An example of this would be the deletion of a "recipient" gene to produce the GMM. In cases of self-cloning, the characteristics of the donor should be provided only when the strain used is different from the recipient. If the donor strain has or belongs to a taxonomic group that has the QPS status, however, no information on this section is needed.

The description of the donor should include:

2.1 Identity

This should include common name, strain designation, information about the source of the strain, accession number from a recognised culture collection, if available.

2.2 Taxonomy

The most detailed description possible should be provided and should include (a) genus, (b) species, (c) subspecies (if appropriate) and (d) strain. The most appropriate taxonomic classification of the organism should be provided. Methods used for the taxonomic identification, to the strain level, should also be provided. The use of the most recent molecular and phenotypic techniques used to establish the identity of the organism is recommended. For fungi, it is important to indicate the teleomorph/anamorph (sexual or asexual) state.

2.3 Other names

When appropriate, the generic name, commercial name, previous name(s), etc. by which the GMM is known should be provided.

2.4 Phenotypic and genetic markers

Phenotypic and genotypic information relevant to identification, genetic stability and/or safety should be provided, not only for the donor strain, but also for related microorganisms, if appropriate. This should include any information relating to pathogenicity, potential immunological impact or human and animal health.

2.5 Description of identification and detection techniques

These should be described in detail. The use of the most recent and reliable molecular techniques is recommended.

2.6 Sensitivity, reliability and specificity of the detection techniques

The choice of detection and identification techniques should be justified and their sensitivity, reliability and specificity, including within-laboratory validation, should be provided.

2.7 Source and habitat of the organism

Information should be provided of the habitat(s) in which the microorganism is found naturally. The source should be specified, whether a wild strain or a commercial strain from a recognised culture collection.

2.8 Pathogenicity traits

Classification of hazard according to the existing Community rules concerning the protection of human health and/or the environment; pathogenicity, infectivity, toxigenicity, virulence, allergenicity, and the ability to act as a carrier of pathogenicity islands should be provided.

2.9 History of use

Information should be provided relating to the past and present use, if any, in food and/or feed and of its unintended presence in food or feed (*e.g.* as a contaminant), if relevant.

3. Description of the genetic modification process

The genetic modification protocol should be described. When helper plasmids are used, they should be described in detail. The use of carrier DNA is discouraged. If, however, 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 microorganism. In some cases, the genetic modification may be achieved by self-cloning. Even in such cases, information on the genetic modification process should be provided. Nevertheless, the requirements to be addressed should be evaluated on a case-by-case basis and they are different when the self-cloning has been achieved within the same strain from cases in which different strains belonging to the same or closely related species are used. A smaller data set will be required in cases in which self-cloning is carried out in microorganisms with a QPS status.

3.1 Characteristics of the vector

The description of the vector should include the following:

a) the nature and source of the vector used: Information should be provided on the DNA used to modify the microorganism, including the description of previous use(s), if available. The copy number for plasmids should be provided. The choice of the vector should be justified and the procedures used to construct it detailed. A physical and genetic map should detail the position of all functional elements and other vector components, together with the applicant's selected restriction endonuclease sites for the generation of probes, and the position and nucleotide sequence of primers used in PCR analysis. A table identifying each component, coding and non-coding sequences, origin(s) of replication and transfer, regulatory elements, their size, origin and role, should accompany the map;

b) the frequency of mobilisation of the inserted vector and its capacity for genetic transfer. Any information on the expected stability of the inserted vector in the recipient 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;

c) information on the degree to which the vector is limited to the DNA that is required to perform the intended function. It is always recommended to avoid or minimise the inclusion of extraneous DNA; all information relating to the host range of plasmid used as a vector should be given.

3.2 Information relating to the genetic modification

The protocol used for the modification should be described in detail; methods used to construct and introduce the insert(s) into the recipient or to delete a sequence(s) from the recipient should be described and justified. Relevant references for the transformation method should be provided, including:

a) a description of the insertion or deletion and/or vector construction: strategies to construct and introduce the insert(s) into the recipient or to delete a sequence(s) from the recipient should be described and justified. Information on the integration site, sequence actually inserted or deleted, on the size and copy number of all detectable inserts, both complete and partial should be provided and methods used for their detection should be detailed and their sensitivity demonstrated. This is typically determined by Southern transfer and hybridisation analysis. Probes used for this purpose should provide complete coverage of sequences that could be inserted into the host microorganism, including all parts of the vector or any carrier or foreign DNA that may remain in the GMM. In general, DNA inserted as a single copy in the chromosome is less likely to be transferred than that present in higher copy number on extrachromosomal elements. In the case of deletion(s), the size and function of the deleted region(s) must be provided. Any polar effects that the deletion event may have on downstream expression should be documented;

b) the nature of the insert: the sequence of the insert or deletion and information on the degree to which the inserted sequence is limited to the DNA required to perform the intended function should be provided. Sequence analysis must extend into regions flanking the modification event. The risk assessment may be simplified if genes not absolutely required for the intended modification of the host are not present in the final GMM;

c) the methods and criteria used for selection: when a marker gene is necessary for the selection of the desired event, careful consideration should be given to the choice in view of the amount of information required for risk assessment and justifications for the choice should be provided. If a gene conferring antibiotic resistance is used, it should be justified and evidence that other marker genes could not be used should be provided. Whenever possible, the use of antibiotic resistance marker genes in GMM construction should be avoided to prevent the possibility that clinical therapy could be compromised. It has been suggested that this may occur by

    (i) inactivation of oral doses of antibiotics from consumption of foods containing live GMMs,
    (ii) the development of resistance to clinically relevant antibiotics in pathogenic microorganisms in the body, and
    (iii) by horizontal transfer of antibiotic resistance genes from GMMs to the resident microbiota in the body which can be a reservoir for subsequent transfer to a pathogen.

Alternative technologies that do not rely on antibiotic resistance marker genes should be used for selection purposes in GMMs;

d) the subcellular location(s) of insert(s) in eukaryotic microorganisms must be determined (*i.e.*, chromosome, chloroplasts, mitochondria or maintained in a non-integrated form) and methods used for this determination must be provided. All information relating to the host range of introduced plasmids should be provided.

4. Identification of the conventional counterpart microorganism and its characteristics

The choice of the comparator microorganism is critical and should be justified. It is important that the applicant be aware that the non-GM counterpart should be the specific non-modified parental or recipient strain, not simply a strain of the species to which the GMM belongs; neither should it be the type strain of the species, unless the type strain is used as the recipient. In most cases, the most appropriate comparator is the parental or recipient strain that is isogenic except for the introduced trait(s). The simplest case is when the comparator and the recipient are derived from the same strain. When they are different, the precise taxonomic identification of the comparator should be provided and its choice must be justified. In any case, the comparator should be a strain with a history of safe use and which has previously gone through safety evaluation. Whenever possible, the comparator should have QPS status. All relevant phenotypic and genotypic traits of the comparator should be described. The methods used to establish the identity of the comparator should be detailed. The most relevant key components (metabolic activity, physiology, safety, etc.) to be considered in the comparative risk assessment should be identified, justified and described. In microorganisms, the presence of mobile genetic elements (plasmids, transposons, integrons and prophage) may lead to natural changes in the genome of the selected comparator strain. Therefore, the genetic stability and variability of the comparator should be demonstrated. A genetic fingerprint using the most recent reliable techniques available should be provided for the comparator to enable its identification and comparison to the GMM. When the comparator belongs to a group of closely related strains, the genetic variability within the group should be demonstrated using molecular techniques. This is important to avoid the attribution of observed differences to the genetic modification when they were already present among the closely related strains. When a history of safe use is not available for the recipient strain, but is for other strains belonging to the same species or genus, the choice of a different strain as comparator should be justified; all the available information should be provided by the applicant and evaluated on a case-by-case basis.

5. Information relating to the GMM and comparison of the GMM with its conventional counterpart

5.1 Description of the genetic trait(s) or phenotypic characteristics and any new trait which can be expressed or no longer expressed

A description of the trait and the changes that it makes to the phenotype of the microorganism is required. Phenotypic differences between the GMM and its comparator should be determined. The applicant should identify whether the GMM differs from its non-GM counterpart in its biology. The purposes of the genetic modification and the uses of the GMM should be described, together with changes in the metabolism of the microorganism. Both qualitative and quantitative differences should be assessed and reported.

5.2 Structure and amount of any vector and/or donor nucleic acid remaining in the final construction of the modified microorganism

The organisation of the inserted genetic material at the insertion site, including sequence information, the location of primers used for detection, and the methods used for the characterisation should be provided. The size and copy number of all detectable inserts, both complete and partial, and the methods used for their detection should be detailed and their sensitivity demonstrated. Applicants should demonstrate that the sequence inserted in the GMM is the one intended. Sequence

determination at both 5' and 3' ends of the inserts should extend into the genome of the recipient. This serves two primary functions. Firstly, it provides information on unique identification sequences for detection purposes (traceability). Secondly, flanking sequence data may identify insertion into, and interruptions of, known ORFs (open reading frames) or regulatory regions and/or the potential for insertional events to produce novel chimeric proteins. Risk assessment of any changes observed should be provided according to the appropriate section of this guidance document. If potential chimeric ORFs are identified, bioinformatic analyses should be conducted to investigate the possibility for similarities with known toxins or allergens. Depending on the information gathered, further analyses may be needed to complete the information necessary for a comprehensive risk assessment. For example, transcriptional and/or translational data may be required to investigate if novel proteins and RNA molecules are synthesised. Thirdly, genomic fingerprints (using PFGE) would be useful to identify any gross genomic changes.

5.3 Stability of the microorganism in terms of genetic traits

The genotype and phenotype of a GMM should be stable over the intended period of production and normal use of the organism in food or feed. The applicant should provide information on potential rearrangement of the modified genetic material that occurred after the modification of the cell and during propagation of the modified strain to the extent needed for its use(s) in food or feed production, including those that may occur during storage. Microorganisms grow fast, adapt to changing environments and are more prone to genetic instability than are higher organisms. Chromosomal and other genetic rearrangements are relatively common compared with higher organisms. The general genetic plasticity of microorganisms and the location of particular transgene(s) may affect the genetic stability of the GMM. The genetic stability of the recombinant microorganism is also dependent on the localisation of the cloned gene(s). Vector systems with a broad host range should be avoided. The stability of the GMM should be demonstrated from several batches and using a variety of growth and storage conditions. Methods used to demonstrate the stability of the GMM should be provided. When instability in the genetic modification could affect safety, evidence relating to the stability should be provided from each batch.

5.4 Rate and level of expression of the new genetic material

The precise function of the new gene product(s), together with a phenotypic description of the new trait(s), should be detailed. The level of expression of the new gene(s) and the location in the GMM of the gene product(s) as well as the expression during growth cycle should be defined. Applicants should provide information on the level of expression of the modified DNA under the conditions envisaged during use of the GMM in food or feed. This information should be derived using several batches. The requirements for information on developmental expression should be considered on a case-by-case basis taking into account the promoter used, the intended effect of the modification and the potential for effects on non-target organisms. Any new substance that may be produced by the GMM and that is not present in its non-GM counterpart should be identified and assessed for risk. The methods used for expression analysis and their sensitivity should be described in detail.

5.5 Description of identification and detection techniques

The techniques used for the identification and detection of the modified sequence and vector should be detailed. The sensitivity, reliability (in quantitative terms) and specificity should be demonstrated and supported scientifically.

5.6 Information on the ability to transfer genetic material to other organisms

Given the ubiquity of natural genetic exchange systems between microorganisms, the transfer of genes from GMMs to other microorganisms can be expected and, therefore, the potential impact of such an event needs to be evaluated. In the construction of GMMs, however, specific measures may be used to reduce the potential for the spread of an introduced recombinant construct or gene. Such measures include the following:

(i) avoiding the use of sequences that could enhance recombination or integration of the introduced trait into the genome of other microorganisms;
(ii) designing chromosomal insertion strategies for the introduced gene and avoiding the use of extrachromosomal replicons and elements;
(iii) avoiding the use of genes in the modified microorganism that could confer a selective advantage to recipient microorganisms in the event of the specific recombinant construct being transferred unintentionally;
(iv) avoiding the use of a broad host-range replicon when the final modification event resides on the vector

In the event that the genetically modified microorganism has the ability to transfer DNA to plants, animals or humans, the control measures to limit such transfer must be identified and supported by experimental evidences.

5.7 Information on the interaction of the GMM with other organisms, when appropriate

The evaluation of potential changes in the interactions of the GMM with other organisms, as compared with the non-GM comparator, must be carried out on a case-by-case basis. This should take into account the biology of the microorganism, the biology of exposed organisms, the characteristics and expression of the introduced genetic material, the properties and consequences of the genetic modification and the scale of release.

5.8 History of previous releases or uses of the GMM, when appropriate

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

5.9 Safety for humans and animals

The risk assessment of the GMM should be based on the overall comparison between the modified microorganism and its conventional non-GM counterpart. Any difference in the metabolic activity, colonisation capacity, and other trait(s) that can affect human and animal health should be defined and assessed for risk. Information dealing with plant and environment health, including interactions with other organisms, and the evaluation of any risk to the receiving environment that might arise from the release of the GMM, are part of the environmental risk assessment (ERA) that is addressed in Section III, D of this document. The risk assessment of the modified microorganism should include the following:

a) information on any toxic, allergenic or other harmful effects on human or animal health arising from the GMM. Studies of pathogenicity appropriate to the GMM must be performed, when relevant. Genes inserted in a GMM should be evaluated for their potential impact on human and animal health. Documented evidence of safe use of the GMM must be provided. The GMM may have been significantly changed

in comparison with its conventional counterpart so that effects on safety must be investigated. Genetic modification may stimulate or de-repress endogenous toxin production so that the GMM should be tested for the production of relevant toxins;

b) assessment of the impact on human and animal health should include the potential for DNA transfer events to take place. It should also take into account any capacity for enhanced gene transfer to occur. Thus, on a case-by-case basis, specific experimental data on gene transfer and its consequences may be required;

c) if the GMM remains viable in the final food or feed, the viability and residence time of the GMM in the alimentary tract should be compared with those of its conventional counterpart (model systems may be used but should be validated). This is particularly important if the viability of the GMM is affected by the genetic modification;

d) information on any impact that the GMM has on the microbiota of the human or animal gastrointestinal tract.

The general population, as well as specific groups which might be particularly vulnerable, should be considered when the safety of a GMM is evaluated. When transformation events have been combined by transfer of existing approved GMM or by re-transformation of an existing GMM, the need for further molecular analysis will depend, on a case-by-case basis, on the nature of the genetic modifications involved. There is no a priori reason to assume that transfer of transgenic material between independent, safe GMMs will pose any additional risk through a compromised stability of copy number and insert structure. Additional unintended effects could arise through the effects of combined genes *e.g.* on biochemical pathways and, on a case-by-case basis, will require appropriate comparative analysis.

5.10 Information on monitoring, control, waste treatment and emergency response plans

Information on monitoring, control, waste treatment and emergency response plans has to be provided, when appropriate. Monitoring strategies and methods for GMMs and relevant recombinant DNA have been addressed in Chapter D.3.

C. INFORMATION RELATING TO THE GM PRODUCT

Information relating to the GM product should include a description of the main characteristics of the product, its intended use(s) and the purpose of the genetic modification. When applicable, a description of the production process and the purification process should be detailed. A comparison with the conventional counterpart should be carried out. Any difference in nutritional properties, chemical composition, physical characteristics or other traits that can affect human or animal health or the environment should be assessed and the safety of the product established.

1. Information relating to the production process

Information relating to the production process of the GMM (fermentation, cultivation) and of the GM product should be provided. The process by which the raw materials are converted into the finished product should be described step-by-step and in detail. The key stages of the production process that may lead to any difference between the GM product and its conventional counterpart should be identified. The parameters most relevant for the characterisation of the product from a safety and nutritional point of

view should be considered. A flowchart showing the key stages is recommended. The applicant should provide the scientific rationale for the risk assessment. Experimental data may be required on a case-by-case basis.

2. Information relating to the product purification process

Information relating to the product purification process should include the description both of techniques used to remove GMM cells and of techniques used to purify the product.

2.1 The technique used to remove microbial cells from the product

The technique used to remove microbial cells from the product should be detailed. The reliability and efficacy of the technique used should be established scientifically. When data from literature are provided, they should come from recent in-depth reviews or papers that have been peer-reviewed. The absence of microbial cells should be established, using both a recognised culture-based method for the enumeration of viable microorganisms, if available, and molecular methods. The use of molecular methods allows the detection of cells that are viable but that cannot be cultivated under laboratory conditions. Different kinds of PCR may be used, using either primers specific for the GM event or primers that can detect a broader group of microorganisms (strain, species, genus or family) to which the GMM belongs. The use of detection techniques with different specificities should be evaluated on a case-by-base basis and should be justified.

2.2 Information on the technique used to kill the microbial cells

Information on the technique used to kill 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. Several techniques may be used to kill cells in a product, and the choice depends principally on the nature of the product. The technique used should be described in detail, justified and all physicochemical parameters adopted should be provided. The reliability, sensitivity, and efficacy of the technique used to kill the specific GMM should be established scientifically, taking into consideration the current literature. There is considerable variability in the resistance of microorganisms to killing agents and methods. For this reason, the efficacy of any technique used to kill GMMs should be established for the specific GMM within the product. The absence of viable GM cells should be verified by means of both a recognised method for determining viable microorganisms, if available, and molecular methods (*e.g.* Real time PCR). When, after killing treatment, viable cells are still present, they should be identified and quantified.

2.3 The process used to purify the product from the microbial growth medium

The process used to purify the product from the microbial growth medium should be described. The extent of purification may vary for different products. The requirements for risk assessment should, therefore, be evaluated on a case-by-case basis. The degree of purity should be expressed in percentage terms and the methods of determination and data used to establish the purity of the product should be provided. The occurrence of impurities should be evaluated and their nature, percentage and methods of determination detailed. In the case of “pure” products, the absence of chemical and microbial impurities should be established.

The potential toxic effects of product processing on food or feed produced using GMMs should be evaluated. The applicant should assess whether or not the processing

and/or preserving technologies applied are likely to modify the characteristics of the end-product compared with its non-GM counterpart. Alterations in the stability of endogenous toxicants or the bioavailability of nutrients can occur as a consequence of the production process. Experimental data may be required on a case-by-case basis.

If no appropriate comparator can be identified, a comprehensive safety and nutritional assessment of the whole product derived from the GMM should be carried out.

3. Description of the product

Food or feed produced from GMMs may include foodstuffs (*e.g.* yoghurts) or their ingredients (*e.g.* amino acids, vitamins, flavouring), food additives (*e.g.* L-cysteine as a flour treatment agent, colourings), feed materials (*e.g.* silage), feed additives (*e.g.* enzymes, vitamins), flavourings, and certain products used in animal nutrition. These may range from single compounds to complex products. It is likely that genetic modification will be used to target pathways resulting in changes in the concentration of non-protein substances or in new metabolites (*e.g.* nutritionally-enhanced foods, functional foods).

The description of the product should include:

3.1 The 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. It should be stated whether the GMMs were removed from the product and whether the product is purified or not.

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.

3.3 Composition

The qualitative and, when possible, quantitative composition of the product, should be provided, including all ingredients and impurities. The extent of batch-to-batch variation should be determined. For products that are single substances, the chemical characteristics (molecular weight, molecular formulae) and the presence and nature of contaminants should be provided. The techniques used to identify the product and to define its chemical composition should be detailed.

3.4 Physical properties

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

3.5 Technological properties

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

4. Assessment of the presence of recombinant DNA and of the potential risk of gene transfer

Even when GMM cells have been killed or removed from the product, the presence of recombinant DNA should be analysed and the likelihood of gene transfer assessed. The presence of recombinant DNA should be assessed using molecular techniques based on the unique sequences that the applicant must provide to detect the transgenic event in question. If recombinant DNA is detected in the product, the applicant should assess any likely risk(s) associated with its transfer from the processed product to other organisms. The technique used to search for the presence of recombinant DNA should be described in detail. The reliability, efficacy and sensitivity of the method should be established.

5. Comparison of the GM product with its conventional counterpart

Comparison of the GM product with its conventional counterpart is the starting point for the risk assessment of the product, either in the case of a single substance, or in the case of a complex food or feed. The conventional counterpart is represented by substances or complex products produced under the same conditions with the involvement of the conventional microorganism. Any identified differences, both intended and unintended, should be assessed regarding their potential impact on human and animal health and on the environment.

The chemical composition of the GM product should be compared with the composition of a conventional counterpart product produced under the same conditions. For single substances, evidence of identity between the chemical structures of the substance derived from the GMM and of its conventional counterpart should be provided by a comparative analysis. When a difference is identified, it indicates that the two substances are not identical, and therefore a full risk assessment of the substance is required.

In the case of complex products and products that are not purified, whether or not they contain viable GMMs, the comparative approach may be more difficult. The chemical composition of the two products (that derived from the GMM and its non-GM counterpart) can be quite different and compounds others than the intended ones may be present in the product derived from the GMM. In this case, it is necessary to identify such compounds as they may have an impact on the nutritional and safety-related characteristics of the product.

Analysis of the key components should include a qualitative and, possibly, quantitative determination. The statistical significance of any observed differences should be assessed in the context of natural variation for each component. Key components to be measured, if the GMM has a significant nutritional impact, are major nutritional constituents (fats, proteins, and carbohydrates), micronutrients (minerals and vitamins) and anti-nutrients such as enzyme inhibitors. Moreover, key toxicants should be screened for in the GM products, although such compounds are usually not produced by microorganisms traditionally used in food processing.

When the comparative analysis is performed using commercial non-GM products corresponding to the GM product, the data used in the comparison may be generated by the applicant and/or compiled from the literature. Databases used for the comparison should be specified. When using data from the published literature, however, they have to be assessed for their quality (*e.g.* type of material analysed, analytical method used, etc.). Ranges as well as mean values should be reported and considered. These data should indicate whether the GM product falls within the natural range of component concentrations found in commercial conventional counterparts. Analytical methods used for the comparative analysis should be detailed and their accuracy, reliability and efficiency established. Moreover, any change in the level of production of metabolites should also be evaluated.

In the case of products from which the GMMs were not removed (fermented food or feed) and when the risk assessment of the GMM used in their production did not highlight any concern, the comparative analysis with the non-GM food or feed counterpart may be restricted and carried out on a case-by-case basis.

When no appropriate comparator can be identified, a comparative risk assessment cannot be made and a more comprehensive safety and nutritional assessment of the products derived from GMMs should be carried out.

6. Considerations for human health and animal health of the GM product

Genes inserted in a GMM should be evaluated for their potential impact on human and animal health. Their impact on the environment is addressed in Section III, D. Assessment of the impact on human and animal health should include the potential for a microorganism to transfer genetic material to other organisms. Thus, specific experimental data on gene transfer and its consequences may be required on a case-by-case basis. When the GM food or feed contains viable GMMs, and when the production process has not been modified as a consequence of the use of the GMM, the first step in the risk assessment of the product should be the comparison of the GMM with its conventional counterpart. This comparison should focus principally on the differences in the metabolic profiles between the GMM and its conventional counterpart growing in the same matrix and in the same product.

6.1 Toxicology

The GMM should not produce toxins including those that may arise unexpectedly as a consequence of the genetic modification event. The requirements for the assessment of human and animal health of food or feed derived from GMMs must be considered on a case-by-case basis, depending on the nature and extent of the introduced or deleted DNA sequence(s). They will be determined by the outcome of the assessment of the differences identified between the GM product and its conventional counterpart, including information available on intended changes. In many cases, the interaction between the GMM metabolism and the growth matrix affects the final composition of the product, and any resultant effect on the safety of the product should be considered. In this case, the risk assessment of the product should focus on the metabolites produced by the GMM during the production process and in the final product. The same approach should be followed for the risk assessment of food or feed when the use of a GMM leads to unavoidable changes in the production process.

Thus, toxicological testing would not only include studies on newly expressed proteins but also the consequences of any genetic modification process (*e.g.* gene silencing or over-expression of an endogenous gene). In principle, the risk assessment must consider the presence of new proteins expressed as result of the genetic modification,

the potential presence of other new constituents and/or possible changes in the level of natural constituents beyond normal variations including fermentation products. Moreover, potential harmful changes in the composition of the microbial population naturally present in the product should be taken into account. The potential deviations from the conventional counterparts may require different toxicological approaches and varying degrees of testing. In some cases, properly designed animal or in vitro studies with the food or feed derived from a GMM may be considered necessary.

When no appropriate comparator can be identified, a comparative risk assessment cannot be made and a comprehensive safety and nutritional assessment of the products derived from the GMM should be carried out. For instance, this would be the case when a trait or traits are introduced with the intention of bringing significant qualitative and quantitative changes in protein or metabolite profiles.

There may be circumstances, when the applicant considers that safety can be reasonably guaranteed 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.

Toxicological studies should be conducted using internationally agreed protocols. Test methods described by the OECD (OECD) or in the most up-to-date Directives on dangerous substances are recommended (EC, 2002d). Use of any methods that differ from such protocols should be justified. Studies should be carried out according to the principles of Good Laboratory Practice (GLP) described in Directive 2004/10/EC (EC, 2004a) and be accompanied by a statement of compliance with GLP.

Toxicology studies evaluating risks to human and/or animal health complement each other. Most studies recommended for the assessment of the safety of the GM food are relevant for the assessment of GM feed. Testing methodologies are essentially the same and the same level of data quality is required. Should specific studies be required to address the efficacy, nutritional value or wholesomeness of GM feed, *e.g.* long-term feeding trials on target species, the information gained could also be used for additional assurance of the safety of the GMM in the case of human consumption.

6.2 Risk assessment of newly expressed proteins

The studies required to investigate the toxicity of a newly expressed protein should be selected on a case-by-case basis, depending on the knowledge available with respect to the source of the protein, its function and activity and its history of consumption by humans or animals. This may require the isolation of the new substance either from the GMM or from the food or feed product. In the case of proteins expressed in the GMM when both the microorganism and the new proteins have a history of safe consumption by humans and animals, specific toxicity testing might not be required.

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

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

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

the methodology used to carry out the search should be specified.
The stability of the expressed protein should be studied under processing and storage conditions and the expected treatment of the food or feed. The influences of temperature, particularly of heat treatments, and pH changes should normally be examined and potential modification(s) of the proteins (*e.g.* denaturation) and/or production of stable protein fragments generated through such treatments should be characterised.

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

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

Subchronic repeated dose oral toxicity studies should be performed, unless reliable information can be provided which demonstrates the safety of the newly expressed protein (including its mode of action) and that the protein is not related structurally and functionally to proteins that have the potential to affect human or animal health adversely. Depending on the outcome of the toxicity studies, additional targeted investigations may be required, including an analysis of immunotoxicity. Where specific legislation is in place, the applicant should follow the guidance given within that framework.
If the applicant considers that a decision on safety can be taken without conducting a repeated dosing study or that other tests are more appropriate, the reason for this decision must be given.

It is essential that the protein used in toxicology tests is equivalent to the newly expressed protein as it is expressed in the GMM. If, due to the lack of sufficient test materials directly extracted either from the GMM or from the food or feed product, a protein is used that was produced from an alternative source, the structural, biochemical and functional equivalence of the substitute protein to the newly expressed GMM protein must be demonstrated. For example, comparisons of the molecular weight, the isoelectric point, amino acid sequence, post-translational modification, immunological reactivity and, in the case of enzymes, the enzymatic activity, are needed to provide evidence for the equivalence.

6.3 Testing of new constituents other than proteins

Identified new constituents other than proteins should be evaluated. This may include toxicological testing on a case-by-case basis. This includes an assessment of their toxic potency and occurrence in the GM food or feed. To establish their safety, information analogous to that described in the “Guidance on submissions for food additive evaluations by the Scientific Committee on Foods”, dealing with both protein and non-protein additives (SCF, 2001a), and Directive 2001/79/EC, Annex, Part I, dealing with additives other than microorganisms and enzymes (EC, 2001b) is needed. This implies the submission of information on a core set of studies and the consideration of whether or not any other type of study might also be appropriate. Normally, the core set includes information on metabolism or toxicokinetics, sub-chronic toxicity, genotoxicity, chronic toxicity or carcinogenicity and reproduction and developmental toxicity.

6.4 Information on natural food and feed constituents

Food and feed constituents comprise a large variety of substances: macro- and micronutrients, secondary metabolites as well as natural toxicants and antinutritional

factors. Some genetically modified microorganisms may be modified in a manner that could result in new or altered level of various metabolites in food or feed produced using these GMMs. When altered metabolite levels beyond natural variation are identified in the product, a detailed risk assessment based on the knowledge of the physiological function and/or toxic properties of these constituents should be submitted. The result of this assessment would determine if, and to what extent, toxicological tests are required. In case of constituents with a physiological or biochemical function (macro- and micronutrients), an integrated toxicological and nutritional assessment is required.

New or altered levels of metabolites produced by a GMM may change the microbial community structure. These possible effects of the use of GMMs for the production of food or feed should be assessed.

6.5 Testing of the whole GM product

If the composition of the GM product is modified substantially, if there is no appropriate conventional comparator or if there are any indications for the potential occurrence of unintended effects, based on the preceding molecular, compositional or phenotypic analysis, not only new constituents, but also the whole product derived from a GMM should be tested. In such cases, the testing programme should include at least a 90-day toxicity study in rodents. Special attention must be paid to the selection of doses and the avoidance of problems of nutritional imbalance. At least two dose levels of the GM and parental test substance should be included in the diet. The highest dose level should be the maximum achievable without causing nutritional imbalance, whilst the lowest level should approximate the anticipated human intake. Stability of test diets and nutritional equivalence between control and test diets are other important aspects to consider (König *et al*., 2004).

Supplemental information on the possible occurrence of unintended effects may be obtained from comparative growth studies conducted with young rapidly growing animal species. Because of their rapid weight gain, such animals are sensitive to the presence of certain undesirable substances in their feed. Studies of this type are, however, limited to those materials suitable for inclusion in their diets and which can be nutritionally matched to a suitable control diet.

The choice of the control diet in testing whole GM food or feed or components derived from the GM food or feed that are compositionally different should be based on the composition of the traditional food or feed or ingredient which is intended to be substituted. The control diet should be informative on whether specific matrix effects may be expected and on the sensitivity of the test system. Whole feeding trials may be run in parallel with experiments in both in vitro and in vivo systems from animal and/or human origin, studying gene expression profiles and/or potential cytotoxicity of newly expressed proteins or metabolites, for instance.

Additional toxicological studies may also be necessary, depending on the potential exposure, the nature and extent of deviation from traditional counterparts and the findings of the feeding study.

Complex genetic modifications involving the transfer of multiple genes, the potential risk(s) of possible interactions between the expressed proteins, new metabolites and original microbial constituents should be assessed. The outcome of the molecular analysis and knowledge of the mode of action of the newly expressed proteins may provide indications for possible synergistic interactions, as well as information on the response to combined administration of proteins to target organisms and regarding effects on the activity of target enzymes.

When GMM constituents, particularly viable cells, are still present in the product, particular attention should be paid to potential interaction(s) with the gut microbiota and the evaluation of any effect on the digestive physiology and immune response of the host.

Any adverse effect(s) noted in individuals exposed to products derived from a GMM as part of their professional activities should be submitted by the applicant.

6.6 Allergenicity

Allergy is an adverse reaction that, by definition, is mediated by the immune system and, particularly, involves IgE antibodies. It affects individuals who have a genetic predisposition (*i.e.* atopic individuals).

This section deals principally with the risks to those individuals when exposed to products derived from GMMs with regard to allergic reactions. Some microorganisms are known to be allergenic and therefore the use of a recipient microorganism that would be known to cause allergic reactions should be assessed, throughout the food chain.

The constituents that are responsible for allergenicity are in nearly all cases proteins. Some protein breakdown products, *i.e.* peptide fragments, may conserve part of the allergenicity of the native protein and thus may be considered as allergens. The specific allergy risk of GMMs is associated

(i) with exposure to newly expressed protein(s) that can be present in the product and
(ii) with alterations to the allergenicity of the whole product, *e.g.* due to over-expression of natural endogenous allergens as an unintended effect of the genetic modification.

The strategies used to assess allergenic risk concentrate on the characterisation of the source of the recombinant protein, the potential of the newly expressed protein(s) to induce sensitisation or to elicit allergic reactions in persons who are already sensitised and whether the transformation may have altered the allergenic properties of the modified food or feed. A weight-of-evidence approach is recommended, taking into account all of the information obtained with various test methods, since no single experimental method yields decisive evidence for allergenicity.

The development of animal models should be encouraged and, once validated, their use may increase the body of evidence to support a conclusion.

6.7 Assessment of allergenicity of newly expressed protein(s)

This should include information on the source of the protein, the amino acid sequence homology comparison and on the resistance to pepsin digestion.

At present, there is no definitive test that can predict the allergenic response in humans to a newly expressed protein.

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. Allergenicity therefore depends upon the genetic diversity and variability in atopic humans. Given this lack of complete predictability, it is necessary to obtain a cumulative body of evidence that minimises any uncertainty with regard to the protein(s) in question, from several steps in the risk assessment process.

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

The source of the transgene must be considered carefully to make clear whether or not it encodes an allergen. Information describing any reports of allergenicity associated with the donor organism, when appropriate, should be provided.

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.

In cases when the introduced genetic material is obtained from wheat, rye, barley, oats or related cereal grains, applicants should assess the newly expressed proteins for a possible role in the elicitation of gluten-sensitive enteropathy or other enteropathies that are not mediated by IgE.

The first step in the assessment should be a search for sequence homologies and/or structural similarities between the expressed protein(s) and known allergens. Identification of potential linear IgE binding epitopes should be conducted by a search for homologous peptidic fragments in the amino acid sequence of the protein. The number of contiguous identical or chemically similar amino acid residues used in the search setting should be based on a scientifically justified rationale in order to minimise the potential for false negative or false positive results. The use of different homology searching strategies based on the sequences available in relevant databases may identify several scenarios. These include a high degree of homology, with or without conservation of the allergenicity, or a low degree of homology with conservation of allergenicity (Mills *et al*., 2003). To reduce the uncertainty of the conclusions that may be drawn from the search of sequence homology alone, efforts should be encouraged to improve the bioinformatic approach, *e.g.*

(i) improve and harmonise the algorithms that are used by the different applicants, and
(ii) develop databases which include information on the three-dimensional structure and function of known allergens and of proteins belonging to protein families which include a high proportion of allergens.

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

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

If the source is not known to be allergenic, but if there are consistent indications of sequence homology to a known allergen, 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 newly expressed protein and this allergen. The results of the screening are interpreted as above. The additional tests that should be performed may include the following:

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

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

As well as targeted screening, 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. The use of existing models and the development and validation of new alternative models that can substitute for and/or complement the use of human biological material for evidence of cross-reactivity and elicitation potency should be encouraged. These approaches would include the search for T-cell epitopes, structural motifs, in vitro cell based assays using animal or humanised-animal immune cells, etc. They also include appropriate *in vivo* animal models.

Animal models are useful tools for the assessment of the sensitising potential of newly expressed proteins, *i.e.* their capacity to induce an allergic immune response with the synthesis of specific IgE in individuals that have never been exposed to those proteins or to proteins that cross-react with them.

6.8 Assessment of allergenicity of the whole GM product

The allergenicity of the whole product may be modulated as an unintended effect of the insertion of the transgene in the genome of the recipient, for example through qualitative or quantitative modifications of the pattern of expression of endogenous proteins. Any potential change in the allergenicity of the whole GM food or feed should be tested by comparison of the allergen repertoire with that of the non-GM food or feed comparator.

These approaches should be applied on a case-by-case basis depending on the available information on the allergenic potential of the source and/or the host. Development of modern analytical tools including profiling techniques may be used in association with human and animal serum or cell-based assays.

Normally this should not be a major issue since most microorganisms are not considered major allergens and possible over-expression of any endogenous protein would be unlikely to alter the overall allergenicity of the whole product.

The integrated process which is described above applies to the assessment of the allergenicity of all the components of GMM products (*i.e.* covers both food and respiratory allergy risk).

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

6.9 Nutritional assessment

6.9.1 Nutritional assessment of the GM food

The development of GM foods may have the potential to improve the nutritional status of individuals and populations and provide products with enhanced functionality. GM foods also have the potential to introduce nutritional imbalances because of both expected and unexpected alterations in nutrients and other food components (ILSI, 2004).

An intended modification introduced in a GMM may alter the overall profile of the product, which, in turn, could affect the nutritional status of individuals consuming the food. The impact of changes that could affect the overall nutrient profile should be determined.

Compositional analysis is the starting point and the cornerstone for the nutritional assessment of food and feed material. It is based on the assessment of possible compositional changes to key nutrients. If such nutritional modifications have been implemented, the product should be subjected to additional testing to assess the consequences of the changes and whether the nutrient intakes are likely to be altered by the introduction of such foods into the food supply.

The biological efficacy of nutrient components in the product should be considered. The analyses conducted should be determined on a case-by-case basis and may vary depending on the introduced trait and on the processing and storage.

An estimation of the expected intake should be provided for a correct evaluation of the nutritional changes.

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

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

The introduction of a significant nutritional change in a food may require post-market assessment to determine if the overall diet has been altered and to what degree (see Section III, C, 6.10).

6.9.2 Nutritional assessment of the GM feed

Once compositional equivalence has been established in GM feeds, nutritional equivalence can be assumed, since routine long-term livestock feeding studies generally add little to a nutritional assessment.

In the case of GM feed with improved nutritional characteristics, such as feed containing a probiotic, livestock feeding studies with target species should be conducted on a case-by-case basis to study the nutritional benefits that might be expected and to provide further safety assurance. These studies should span either the growing and/or finishing period to slaughter for chickens, pigs, and cattle for fattening or a

major part of a lactation cycle for dairy cows and should be conducted according to internationally agreed standard protocols, when available. For feedstuffs intended only for aquaculture, growth studies with fish species such as carp may be preferable to an extrapolation from results obtained with land animals.

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

When studies are conducted, the following aspects should be considered:

a) GM feeds (feeds to which GMM-derived components have been added) modfied for improved bioavailability of nutrients: livestock studies with target species should be conducted to determine the bioavailability of individual nutrients in the GM feed and a range of commercially available feeds with similar nutritional composition;

b) GM feeds specifically modified with traits to enhance animal performance through increased nutrient density or an enhanced level of a specific nutrient: an appropriate control diet using its nearest genetic counterpart should be formulated by supplementing it with the specific nutrient to the extent of the change effected in the GM feed. It is also suggested that a number of other commercially similar feeds may be included in the study;

c) GM products from which the modified ingredient has been extracted should be compared with those derived from an appropriate counterpart and other commercial similar feeds on the basis that they are essentially free from the modified component;

d) Considering future developments, attention is drawn to the potential effect of GM feeds with modified nutritional value on the composition of foods derived from animals fed these GM feeds.

6.10 Post-market monitoring of GM products

When appropriate, a post-market monitoring programme (PMM) should be developed for the GM product. For instance, if the product contains viable cells of a GMM for which no conventional counterpart can be identified and that may interact with the gut microbiota and have an effect on the physiology of the host, the effects on the human and animal health could be difficult to predict in the pre-market risk assessment. In this case, a PMM plan is recommended.

PMM does not substitute for a thorough pre-marketing toxicological testing programme but complements it in order to confirm the pre-market risk assessment. It may increase the probability of detecting rare unintended effects. Therefore, the PMM for GM foods should be designed to generate reliable and validated flow of information between the different stakeholders, which may relate GM food consumption to any (adverse) effect on health.

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

(i) is the use of the product as predicted or recommended?
(ii) are known effects and side-effects as predicted? and
(iii) does the product induce unexpected side-effects?

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

D. POTENTIAL ENVIRONMENTAL IMPACT OF GMMs AND DERIVED PRODUCTS

The potential environmental impact of GMMs used for the production of food or feed or food or feed consisting of or containing GMMs should be analysed on a case–by-case basis. When appropriate, potential adverse effects on the receiving environment should be evaluated whether direct or indirect, immediate or delayed, as a consequence of the deliberate or accidental release or the placing of GM food or feed on the market.

The approach for the environmental risk assessment of products derived from GMMs is indicated in the flow diagram in Figure 1.

When appropriate, the GMM should be compared with the non-GM comparator.

For **Group 1:** Single compounds or defined mixtures derived from GMMs that do not contain functional recombinant DNA, no environmental risk assessment has to be done.

For **Group 2:** Complex products derived from GMMs but not containing viable GMMs nor unit length of any cloned (foreign) open reading frames, the environmental risk assessment is restricted to cases where the product contains recombinant DNA. The potential for transfer of this DNA by transformation and its possible consequences should be assessed. See Section III, C, 4.

For **Group 3:** GMMs and products containing viable GMMs or genetically intact cloned (foreign) DNA, a complete environmental assessment is needed, even in case in which self-cloning was used to obtain the recombinant microorganism. This assessment is conducted at two levels. Level 1 is needed for all GMMs of this group, while level 2 is an additional level for GMMs assessed to have access to the environment as a metabolically-active entity.

1. Environmental Assessment for Level 1 cases

1.1 Spread of the GMM from the product to external environments

Assessment of spread from the food or feed of the GMM into external environments should be based on the density of the GMM in the different compartments involved in its handling (*e.g.* inoculum, food or feed, waste, faeces and manure) and the scale of the different activities. During this assessment, it is important to consider:

(i) direct transmissions to the environment during handling of the GMM, handling of the food or feed, handling of waste and deposition of faeces in the external environment by humans or animals; such transmissions might be affected by movements of air and water, drainage systems, handling of livestock and products;

(ii) indirect transmissions to the environment by waste disposal, uses of manure and slurry as fertilisers in fields, spill and wastewater from sewage plants, deposition and use of sludge and unintended uses of the GMM;
(iii) accidental releases of the GMM into the environment.

It is also important to consider how likely the transmission from the site of use to a specific external environment will be, how far the GMM can be moved and whether it can survive long enough to reach the specific environment. During the assessment, environments that act as potential recipients should be identified. GMMs that are not spread to external environments, or are unable to spread, need no further evaluation.

1.2 General ability of the GMM to survive and persist in external environments

The extent to which the GMM can survive and persist in the environment is an important and highly relevant issue in any environmental risk assessment. The possible impact will be significantly reduced if the GMM cannot survive in the external environment to which it may gain access. The "external environment" is here regarded as environments external in relation to the food or feed and the gastrointestinal tract of the humans or animals consuming the food or feed. Therefore, the ability of the GMM to survive as a metabolically active entity and multiply outside the food or feed needs to be evaluated. This evaluation should be based on both the general physiological traits of the GMM, and the likely effects of the insert on its fitness in these external environments. The points to consider in this assessment include specific requirements and limiting factors for growth, consideration of the possible survival strategies of the GMM in these external environments and during adverse conditions, the occurrence of specific survival structures such as spores and non-specific structures such as minicells, viable but non-cultivable cells, etc. The traits of the GMM that may confer resistance to natural control factors, such as antibiotics, bacteriocins, bacteriophage, etc. need to be included in the evaluation of the survival ability of the GMM. GMMs with no ability to survive in external environments need no further evaluation.

1.3 Transfer of recombinant DNA

If no viable GMMs are expected to be released to the environment, the possible transfer of the recombinant DNA by transformation and its possible consequences in the environment should be assessed. See Section III, C, 4.

2. Environmental assessment for Level 2 cases

Potential receiving environments for the GMM should be characterised and the transmission route to the environment described. The concentration of the GMM in the material reaching the environment needs to be reported and the amount of material to be found in the receiving environment needs to be calculated. The characterisation should include a description of key factors of importance for growth and survival as well as potential growth limiting factors for the GMM in these environments; such factors include temperature, pH, water tension, availability of organic carbon and macro- and micro-nutrients, root-growth, competitors, predators, etc. Any factor(s) that may confer a selective advantage upon the GMM in these environments need to be described.

Any characteristic of the GMM linked to the genetic modification event that may result in effects on the potential receiving environment(s) should be identified. These may include target and non-target effects. A comparison of the characteristics of the GMM with those of its conventional counterpart under the same release conditions should be considered. Any identified difference that may have effects on the environment

should be analysed and assessed for risk, on a case-by-case basis. The following points should be addressed, when appropriate:

2.1 The potential for survival in receiving environments and selective advantage

The number of GMMs transmitted into a specific external environment and their potential for survival are important factors to consider in the environmental risk assessment. Potential effects on the receiving environment are dependent on the survival of the microorganism either as viable or viable but non-cultivable cells. If the GMM has a higher potential for survival than its conventional counterpart, then it is more likely to have an environmental impact. An assessment is required of the likelihood of the GMM to have a higher potential for survival in any of the identified potential receiving environments. For this purpose, data from laboratory experiments in micro- or mesocosms or from small-scale field releases will be important. In such assessments, the key factors for survival competition and growth need to be identified for the specific environment.

2.2 The potential for transfer of recombinant genes

Gene transfer may occur between the GMM and indigenous microorganisms in the environment. Release of a GMM into a specific environment may result in gene transfer through conjugation, transduction or transformation (by homologous or non-homologous recombination). Several conditions must be met if gene transfer is to occur and have an impact in an ecological context. First, the population density of the donor and recipient organisms must be sufficiently high to ensure that the transfer is probable within the given spatial and temporal conditions. Second, if the gene(s) is transferred it must be functional in the recipient. Third, the expression of the gene(s) that is transferred confers a selective advantage or enhanced fitness on the recipient microorganism. Information on gene transfer obtained through experiments in laboratory systems (micro- or mesocosms) may be used to assess the likelihood and the extent of gene transfer from GMMs. However, it is extremely difficult to predict the occurrence of gene transfer events in complex environments. Therefore, genetic constructs should be designed in ways that minimise the potential for gene transfer, in order to make it possible to predict minimum exposure and therefore reduced risk. Additional care should be taken if gene transfer may result in significant increase in fitness or selective advantage of the resident organisms in the specific environment.

2.3 Effects on indigenous microorganisms

The GMM may displace or otherwise affect particular component(s) of the indigenous microbial community negatively after transmission to a specific environment. Such a displacement may be caused by high persistence or competitive ability of the GMM in the environment and/or the production of toxic compounds. The extent and duration of the displacement will also depend on the number of GMM cells reaching the environment, the method of transmission to the environment and the environmental conditions in which the GMM is found. Displacement effects should be considered in relation to functional microbial diversity. For example, consideration should focus on whether displacement could affect key microbial species involved in nutrient cycling, beneficial plant-microbe interactions or degradation of recalcitrant molecules. If the comparative analysis between the GMM and the non-GM counterpart indicates that the transmission of the GMM to a specific environment might disrupt vital processes mediated by indigenous microorganisms, then additional experimentation needs to be done to assess the consequences of these effects.

2.4 Effects on humans

Occupational exposure to the GMM by individuals is likely to be greater compared with the average exposure of the human population, although individuals who consume large quantities of a particular food may have a greater exposure. Potential routes of exposure for workers must therefore be identified in order to evaluate the risk of disease or damage in accordance with the knowledge of the use of the GMM. In this connection, the identified sources of exposure should be weighed against the routes of exposure to the GMM. The applicant should determine the route(s) by which the microorganism is 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 GMM has the ability to enter or to be taken up by the human body and, if this is the case, by which routes. If one or more routes of exposure and relevant routes of entry are identified, the probability of disease or damage should be evaluated. In particular, emphasis needs to be placed on whether the GMM can cause disease or damage in situations during its use, either by colonisation, infection or by production of harmful substances, such as toxins, allergens or carcinogens. When appropriate, quantitative methodologies relevant for human exposure assessment should be adopted (SSC, 2003a).

2.5 Effects on animals

When appropriate, exposure of relevant animals (including both vertebrates and invertebrates) to the GMM and its products or derivatives should be evaluated, and potential harmful effects should be assessed on a case-by-case basis.

2.6 Effects on plants

When appropriate, exposure of relevant plants to the GMM should be evaluated and potential harmful effects should be assessed on a case-by-case basis. Further, if appropriate, it should be assessed whether the GMM can stimulate the growth of certain plant species and affect their weediness after transmission to a specific environment.

2.7 Effects on biogeochemical processes

When appropriate, an assessment is required of the possible effects on biogeochemical processes resulting from potential direct or indirect interactions of the GMM or its products or derivatives after transmission to a specific environment. Microorganisms play an essential role in biogeochemical cycles, such as those of carbon, nitrogen, phosphorus, sulphur and trace elements. Some GMMs might increase the supply of such elements, particularly if their mode of action is to increase the availability of limiting elements in food or feed. However, microorganisms can also reduce the availability of elements by volatilization, oxidation/reduction, by immobilisation or sequestration.

When appropriate and taking into account the population density of the GMM, the applicant should address the potential impacts on biogeochemical processes (such as soil respiration, N-mineralisation, ammonia oxidation, denitrification, turnover of organic matter) as these influence the ecosystem function in the specific environments where the GMM might have access. This should be assessed on a case-by-case basis with particular reference to the host strain (whether it is indigenous to the environment or not), and the nature of the introduced trait.

The assessment should also address the fate of any (newly) expressed gene products and derivatives in those environments where they are transmitted and results in exposure of non-target organisms. Exposure to relevant biota in the environment should also be estimated in relation to impact on decomposition processes.

3. Environmental Monitoring Plan

3.1 General

Regulation (EC) 1829/2003 (EC, 2003a) 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) 1829/2003 Art. 5(5)(b) and Art 17(5)(b)) and a proposal for the post-market monitoring regarding use of the food and feed for human and animal consumption (Regulation (EC) 1829/2003 Art. 5(3)(k) and Art. 17(3)(k)). The latter is not described in any detail in the Regulation (EC) 1829/2003. Section III, C, 6.10 of this Guidance refers to the post-market monitoring of GM food or feed.

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

Since Regulation (EC) 1829/2003 refers explicitly to Annex VII of Directive 2001/18/EC the structure and content of this environmental monitoring plan should be designed in accordance with the Council Decision 2002/811/EC supplementing Annex VII (strategy, methodology, analysis, reporting; EC, 2002b, see also ACRE, 2004; Wilhelm *et al.*, 2003).

An environmental monitoring plan is required for applications for placing on the market of GMOs or food or feed containing or consisting of GMOs conforming with Annex VII to Directive 2001/18/EC. The Guidance notes supplementing Annex VII explain that the extent of the market release shall be taken into account. Thus, the monitoring plan should be targeted rather than considering every possible environmental aspect.

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

3.2 Interplay between environmental risk assessment and monitoring

3.2.1 Monitoring of effects: foreseen and unforeseen

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

3.2.2 Monitoring framework

Council Decision 2002/811/EC (EC, 2002b) explicitly suggests that general surveillance should include long-term monitoring, to allow for unexpected effects that may occur after longer periods of environmental exposure. The environmental monitoring plan should describe in detail the monitoring strategy, methodology, analysis, reporting and review as laid down in Council Decision 2002/811/EC. In this respect,

a) GMM-based parameters will depend on the particular GMM, trait and environment combination. Key parameters to be observed may refer to biodiversity and functionality of species or ecosystem. Indicators should be measurable, appropriate, adequate in terms of statistical power, and comparable with existing baseline data.

b) background and baseline data, *e.g.* relevant environmental parameters, climatic conditions, general application management data should be collected, when appropriate, to permit the assessment of the relevant parameters listed under (a).

3.3 Case-specific GM monitoring

The main objective of case-specific monitoring is to determine the significance of any potential adverse effect identified in the risk assessment (see Sections III, D, 1, 2). The assessment of risk should be based on Annex II of the Directive 2001/18/EC.

Case-specific monitoring should be targeted at those environmental factors most likely to be adversely affected by the GMM that were identified in the environmental risk assessment. The scientific approach should be designed in order to test the specific hypothesis of potential adverse effects derived from the environmental risk assessment. In order to monitor potential risks identified in the risk assessment, environmental hotspots may be identified, in which the effect is most likely to occur and/or in which the GMM food or feed is likely to end up. The monitoring programme design should also reflect levels of exposure and other specific influences. The scale of the monitoring should be increased as the GMM exposure increases. The monitoring should consist of the systematic recording of relevant parameters at representative locations and hotspots. The methods selected, the duration of the monitoring, the extent and the parameters to be monitored will be determined on a case-by-case basis. Whilst the planning and execution of case-specific monitoring is the responsibility of the applicant, it may be appropriate for the applicant to involve public institutions to contribute to the agreed work.

3.4 General surveillance of the impact of the GMM

General surveillance is always routinely applied even in circumstances in which no adverse effect has been identified in the risk assessment. It is required in order to detect unforeseen or unanticipated adverse effects. Monitoring of potential adverse cumulative long-term effects is an important objective of monitoring (EC, 2002b). Potential adverse cumulative and/or long-term effects of the GMM identified in the risk assessment should be considered initially within case-specific monitoring.

One of the objectives of the Directive 2001/18/EC (EC, 2001a) is to protect the environment, including biodiversity, water, and soil. Recently, EU Directive 2004/35/EC on environmental liability with regard to the prevention and remedying of environmental damage (EC, 2004c) defined environmental damage as a measurable adverse change in a natural resource or measurable impairment of a natural resource service, which may occur directly or indirectly.

A major challenge of general surveillance is determining whether:

(i) an observed effect is unusual;
(ii) an unusual effect is adverse; and
(iii) the adverse effect is associated with the GMM or its use.

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

3.4.1 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 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.

In many cases, unforeseen effects of a GMM can only be addressed by looking at general aspects (such as ecosystem functioning on a broad scale). It will be impossible to address all receiving environments, and therefore the applicant should focus, whenever possible, on those environments where the exposure is greatest. The applicant needs to consider assessing possible changes in ecosystem functioning and provide a strategy to detect these changes.

General surveillance plans should be developed for all GMMs that have the potential to enter and survive in the environment. Existing surveillance systems should be used where practical (*e.g.* routine recording systems), and any 'unusual' observations, not occurring in similar reference situations, should be recorded.

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

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

If unusual observations on human health and the environment are reported, more focussed in-depth studies should be carried out in order to determine cause and relationship with the GMM. Such additional case-specific monitoring studies would require an appropriate experimental approach to confirm the specific factor(s) associating an observed effect 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.

3.4.2 Main elements of General Surveillance

The applicant should

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

3.5 Monitoring systems

General surveillance could, when compatible, make use of established surveillance practices. 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. In some cases user surveys might be a useful approach to collecting first hand data on the impact of a GMM on receiving environments. There should be emphasis on the statistical design and representativeness of these surveys. 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).

In many cases, meaningful general surveillance is difficult to achieve and, therefore, currently, it is not possible to provide guidance that is more specific.

3.6 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.

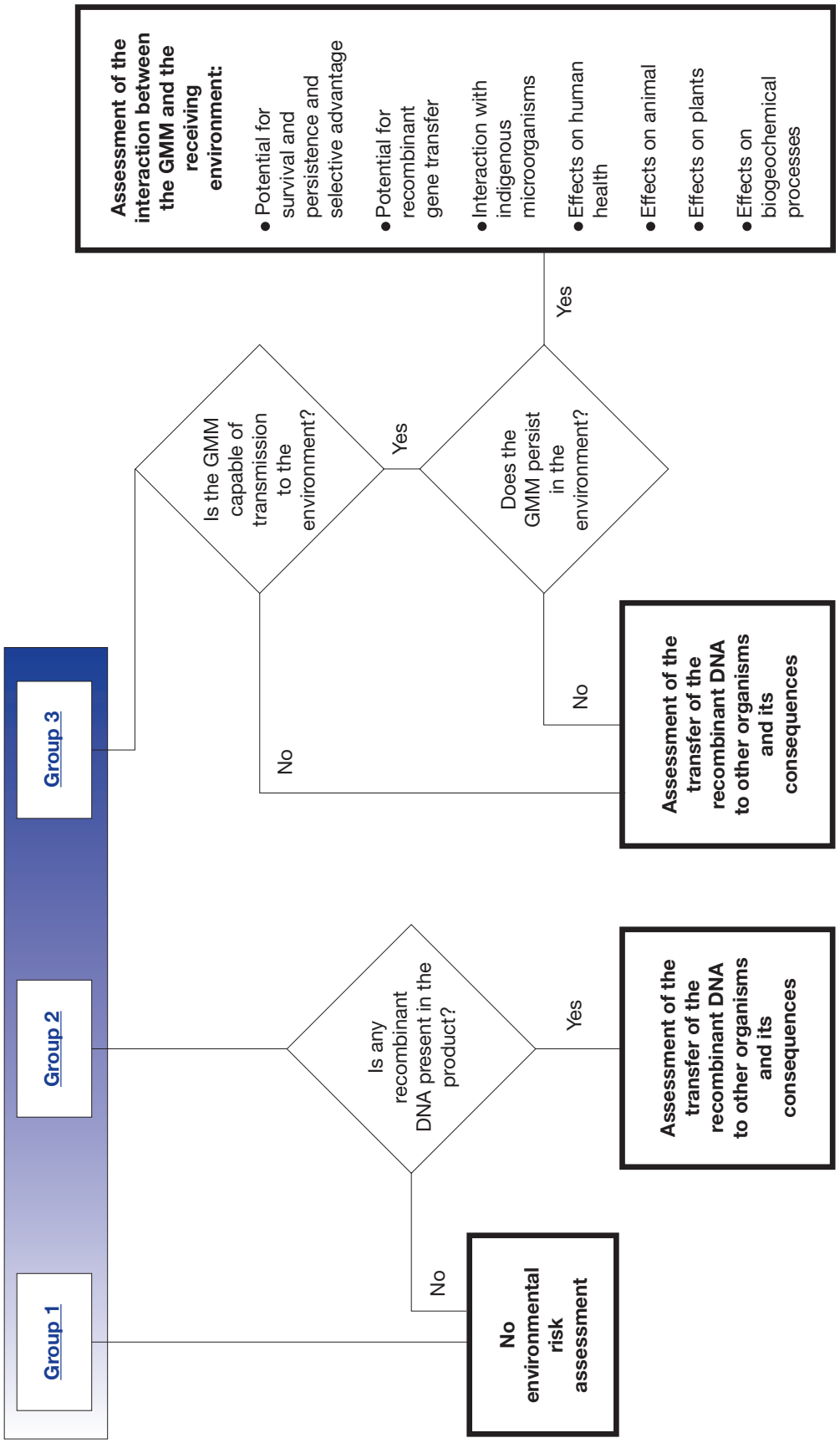

Figure 1. Flow diagram showing the approach to the environmental risk assessment of GMMs and their products

E. SUMMARY OF THE RISK ASSESSMENT REQUIREMENTS

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

This table, based on the approach described in Chapter II, 2 and in Figure 1, contains the main items required to the risk assessment of GMMs and derived food and feed with cross-references to 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.

	Group 1	Group 2	Group 3	Chapter, paragraph
Characteristics of the recipient or parental microorganism				III, B, 1
1. Identity	X[a]	X[a]	X[a]	III, B, 1.1
2. Taxonomy	X[a]	X[a]	X[a]	III, B, 1.2
3. Other names	X[a]	X[a]	X[a]	III, B, 1.3
4. Phenotypic and genetic markers		X[a]	X[a]	III, B, 1.4
5. Degree of relatedness between recipient and donor(s)		X[b]	X[b]	III, B, 1.5
6. Description of identification and detection techniques		X[a]	X[a]	III, B, 1.6
7. Sensitivity, reliability and specificity of the detection techniques		X[a]	X[a]	III, B, 1.7
8. Source and natural habitat			X[a]	III, B, 1.8
9. Organisms with which transfer of genetic material is known to occur		X	X	III, B, 1.9
10. Information on the genetic stability		X	X	III, B, 1.10
11. Pathogenicity, ecological and physiological traits		X[a]	X[a]	III, B, 1.11

(a) Information not required if proposed QPS status is authorised
(b) Information not required in case of self-cloning within the same strain

	Group 1	Group 2	Group 3	Chapter, paragraph
12. Information on indigenous mobile genetic elements	X[a]	X[a]	X[a]	III, B, 1.12
13. Description of its history of use		X	X	III, B, 1.13
14. History of previous genetic modifications	X	X	X	III, B, 1.14

	Group 1	Group 2	Group 3	Chapter, paragraph
Characteristics of the donor organism(s) [a, b]				III, B, 2
1. Identity	X	X	X	III, B, 2.1
2. Taxonomy	X	X	X	III, B, 2.2
3. Other names	X	X	X	III, B, 2.3
4. Phenotypic and genetic markers		X	X	III, B, 2.4
5. Description of identification and detection techniques		X	X	III, B, 2.5
6. Sensitivity, reliability and specificity of the detection techniques		X	X	III, B, 2.6
7. Source and habitat of the organism			X	III, B, 2.7
8. Pathogenicity traits		X	X	III, B, 2.8
9. History of use	X	X	X	III, B, 2.9

(a) Information not required if proposed QPS status is authorised
(b) Information not required in case of self-cloning within the same strain

	Group 1	Group 2	Group 3	Chapter, paragraph
Description of the genetic modification process				III, B, 3
1. Characteristics of the vector		X	X	III, B, 3.1
2. Information relating to the genetic modification	X	X	X	III, B, 3.2

	Group 1	Group 2	Group 3	Chapter, paragraph
Identification of the conventional counterpart microorganism and its characteristics			X	III, B, 4

	Group 1	Group 2	Group 3	Chapter, paragraph
Information relating to the GMM and comparison of the GMM with its conventional counterpart				III, B, 5
1. Description of the genetic trait(s) or phenotypic characteristics and any new trait which can be expressed or no longer expressed	X	X	X	III, B, 5.1
2. Structure and amount of any vector and/or donor nucleic acid remaining in the final construction of the modified microorganism	X	X	X	III, B, 5.2
3. Stability of the microorganism in terms of genetic traits		X	X	III, B, 5.3
4. Rate and level of expression of the new genetic material			X	III, B, 5.4

	Group 1	Group 2	Group 3	Chapter, paragraph
5. Description of identification and detection techniques	X	X	X	III, B, 5.5
6. Information on the ability to transfer genetic material to other organisms		X	X	III, B, 5.6
7. Information on the interaction of the GMM with other organisms			X	III, B, 5.7
8. History of previous releases or uses of the GMM	X	X	X	III, B, 5.8
9. Safety for humans and animals	X	X	X	III, B, 5.9
10. Information on monitoring, control, waste treatment and emergency response plans			X	III, B, 1.10

	Group 1	Group 2	Group 3	Chapter, paragraph
Information relating to the production process	X	X	X	III, C, 1

	Group 1	Group 2	Group 3	Chapter, paragraph
Information relating to the product purification process				III, C, 2
1. Technique used to remove microbial cells from the product	X	X		III, C, 2.1
2. Information on the technique used to kill the microbial cells	X	X		III, C, 2.2
3. Process used to purify the product from the microbial growth medium	X	X		III, C, 2.3

	Group 1	Group 2	Group 3	Chapter, paragraph
Description of the product				III, C, 1
1. Designation of the product	X	X	X	III, C, 3.1
2. Intended use and mode of action	X	X	X	III, C, 3.2
3. Composition	X	X	X	III, C, 3.3
4. Physical properties	X	X	X	III, C, 3.4
5. Technological properties	X	X	X	III, C, 3.5

	Group 1	Group 2	Group 3	Chapter, paragraph
Assessment of the presence of recombinant DNA and of the potential risk of gene transfer	X	X	X	III, C, 4

	Group 1	Group 2	Group 3	Chapter, paragraph
Comparison of the GM product with its conventional counterpart	X	X	X	III, C, 5

	Group 1	Group 2	Group 3	Chapter, paragraph
Considerations for human health and animal health of the GM product				III, C, 6
1. Toxicology	X	X	X	III, C, 6.1
2. Risk assessment of newly expressed proteins	X	X	X	III, C, 6.2
3. Testing of new constituents other than proteins	X	X	X	III, C, 6.3
4. Information on natural food and feed constituents	X	X	X	III, C, 6.4
5. Testing of the whole GM product	X	X	X	III, C, 6.5
6. Allergenicity	X	X	X	III, C, 6.6
7. Assessment of allergenicity of newly expressed proteins	X	X	X	III, C, 6.7
8. Assessment of allergenicity of the whole GM product	X	X	X	III, C, 6.8
9. Nutritional assessment	X	X	X	III, C, 6.9
10. Post-market monitoring of GM products		X	X	III, C, 6.10

	Group 1	Group 2	Group 3	Chapter, paragraph
Potential environmental impact of GMMs and derived products				III, D

	Group 1	Group 2	Group 3	Chapter, paragraph
Environmental assessment for level 1 cases				III, D, 1
1. Spread of the GMM from the product to external environments			X	III, D, 1.1
2. General ability of the GMM to survive and persist in external environments			X	III, D, 1.2
3. Transfer of recombinant DNA		X	X	III, D, 1.3

	Group 1	Group 2	Group 3	Chapter, paragraph
Environmental assessment for level 2 cases				III, D, 2
1. The potential for survival in receiving environments and selective advantage			X	III, D, 2.1
2. The potential for transfer of recombinant genes			X	III, D, 2.2
3. Effects on indigenous microorganisms			X	III, D, 2.3
4. Effects on humans			X	III, D, 2.4
5. Effects on animals			X	III, D, 2.5
6. Effects on plants			X	III, D, 2.6
7. Effects on biogeochemical processes			X	III, D, 2.7

	Group 1	Group 2	Group 3	Chapter, paragraph
Environmental monitoring plan			X[c]	III, D, 3

(c) Required only for those GMMs which persist in the environment

IV. RISK CHARACTERISATION OF GM MICROORGANISMS REGARDING FOOD OR FEED SAFETY AND ENVIRONMENTAL IMPACT

1. Introduction

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

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

An extensive overview of risk assessment procedures is provided by the Scientific Steering Committee of the European Commission (SSC, 2000; 2003b) and by ILSI (ILSI, 2003). A detailed strategy for risk assessment and risk characterisation of foods derived from GMMs has recently been described by FAO/WHO (WHO/FAO, 2001b), for chemicals in food and diet by Food Safety in Europe (FOSIE, 2002; 2003), and for environmental risk assessment by the EU (EC, 2002a). Guidelines for the risk assessment of foods derived from GMMs were published by Codex Alimentarius (*Codex Alimentarius,* 2003).

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

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

2. How to carry out the risk characterisation

Risk analysis starts with defining the proper questions that should be addressed during the risk assessment, *i.e.* identification of potential risks of preparation of pure cultures of the GMM and human or animal consumption of derived foods or feed, and how these questions should be addressed. Problem formulation should involve risk managers, risk assessors and stakeholders *e.g.* producers, environmental and consumer groups. For instance, production processes, intake and exposure routes, population targets (humans, animals or the environment) and health end-points should be identified for the GMM and its derived food or feed and existing knowledge on the use of the non-modified counterpart and derived food or feed should be collected.

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

The risk characterisation should provide evidence whether the hazard identification and subsequent characterisation is complete. It is essentially an iterative process. Integration and evaluation of data from hazard characterisation and exposure assessment may indicate that an appropriate risk estimation can be made, or that further data should be generated in order to complete the risk characterisation. For instance, if an increased intake of a food or feed derived from a GMM by humans or animals may be expected, further data on toxicity at extended dose ranges may have to be generated.

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

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

The absence of data essential for the risk assessment should be indicated and the quality of existing data should be discussed. It should be clear from the discussion how this body of information has been taken into account when the final risk estimation is determined.

Risk estimation may be qualitative and, if possible, quantitative depending on the issue to be addressed and the available data. The terms for the expression of risks and associated uncertainties should be as precise as possible. For instance, expressions like ‘negligible/acceptable/significant risk’ need, if possible, further numerical quantification in terms of probability of exposure and/or occurrence of adverse effects.

3. Issues to be considered for risk characterisation

Risk characterisation of GMMs should be carried out in a holistic manner as stated above and on a case-by-case basis depending on the type of product derived from the GMM, 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 step. The list of issues is by no means exhaustive.

Molecular characterisation

Evaluation of the characteristics and previous use of the recipient 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 microorganism which may be unintentionally increased as result of the genetic modification.

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

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

Comparative analysis

An important issue to be evaluated is whether the comparative analysis between the GMM and its non-GM counterpart with respect to phenotypic and genotypic characteristics has been carried out appropriately according to current guidelines. It is also important to consider what body of knowledge is available regarding the conventional counterpart product so that it may be taken as a reference for safe human or animal use. Protocols for and performance of analysis should be evaluated, and the data generated assessed to confirm they are representative for the proposed use of the GMM and its derived product.

The goal of the comparative risk assessment is to identify possible differences between the GMM and its conventional counterpart. The choice of the comparator is a key consideration; both for the GMM and for derived products, and its use should be justified. The risk characterisation should concentrate on statistically significant differences in the physiology, biology, metabolic activity and genetic characteristics of the GMM compared to its non-GM counterpart and whether these differences are likely to have an environmental, and/or food or feed safety or nutritional impact. The same approach should be followed for the comparison of the GM product with its conventional counterpart. Moreover, an analysis should be made of the uncertainties associated with the comparative analysis.

Another important issue to be addressed is whether, besides intended effects, unintended effects may occur as result of the genetic modification. The strategy for detection of unintended effects should be discussed, particularly with respect to the probability that significant unintended effects have been missed. When the occurrence of unintended effects cannot be excluded, strategies to assess the potential human or animal health and environmental implications should be explained.

Food and feed safety in relation to intake

The data generated to estimate possible risks to human or animal health associated with the consumption of foods or feed derived from a GMM should be evaluated with respect to the expression of new proteins or metabolites as well as significantly altered expression of original microbial proteins or metabolites in GMM and of whole GM food or feed. Dose response relationships, threshold levels, delayed onset of adverse effects, risks for certain groups in the population, use of uncertainty factors in extrapolation of animal data to humans should be presented.

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

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

Data provided to assess the allergenic potential of newly expressed proteins in GMMs should be evaluated with respect to a possible provocation of allergic reactions of susceptible individuals. Information is also required to demonstrate that the genetic modification process does not cause unwanted changes in the characteristics and/or levels of expression of endogenous allergenic proteins in the food derived from a GMM. In particular, the test models used should be discussed with respect to specificity, predictability and validation status.

With respect to intake estimations of foods for humans 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. For the GM products in questions the requirement for post-market monitoring should be discussed as a mechanism necessary for determining changes to overall dietary intake patterns of the GM food, to what extent this has occurred and whether or not the product induces known (side) effects or unexpected side-effects. If the performance of post-market monitoring is deemed necessary, the reliability, sensitivity and specificity of the proposed methods should be discussed.

Environmental impact

Predicting impacts of GMMs and derived food or feed on complex ecosystems that are continually in flux is difficult and largely based on experiences with other introductions and an understanding of the robustness of ecosystems. It is recognised that an environmental risk assessment is limited by the nature, scale and location of experimental releases, which environments have been studied and the length of time the studies were conducted. The likelihood of transmission of the GMM from the product to the environment and the likelihood of the GMM for survival and persistence in the external environment, as well as the possibility of transfer of recombinant DNA from the GMM and/or its derived product to other organisms are the key points to be considered in the environmental impact evaluation. Evaluations should be conducted against the background of hazards likely to be encountered. Probabilistic methods could be used to determine ranges of plausible values rather than single values or point estimates, which are subsequently combined in order to quantify the uncertainty in the end result. These methods could provide a powerful tool to quantify uncertainties associated with any steps in the risk assessment.

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

Scientific knowledge and experience gained from placing on the market of food or feed derived from a GMM during the monitoring and provisional approval periods for GMM products will also inform the risk assessment process and are opportunities to update environmental risk assessments continually in the light of any new knowledge.

4. The result of risk characterisation

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

When a scientific risk assessment cannot be completed because of the lack of essential data or the availability of poor quality data, this should be indicated.

The risk characterisation should include:

- whether placing on the market of a GMM and its derived products is as safe for the environment as the placing on the market of the equivalent non-GMM;
- whether consumption of food or feed derived from GM microorganisms is as safe for humans or animals as the conventional counterparts;
- specific conditions for production process of food and feed derived from a GMM, if required;
- the scientific basis for different options to be considered for risk management.

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Annex I

EFSA Guidance to applicants on the presentation of applications for the request of authorisation of genetically modified microorganisms and their derived products intended for food and feed use

Introduction

This annex provides guidance on the presentation of applications for the placing on the market of genetically modified microorganisms and their derived products intended for food and feed use introduced under Community legislation (on genetically modified (GM) food and feed[10] and on the deliberate release into the environment of genetically modified organisms[11] (GMOs)) to be evaluated by the GMO Panel of EFSA. This annex will be regularly updated in view of the experience that EFSA and the GMO Panel will develop with the handling of GMO applications.

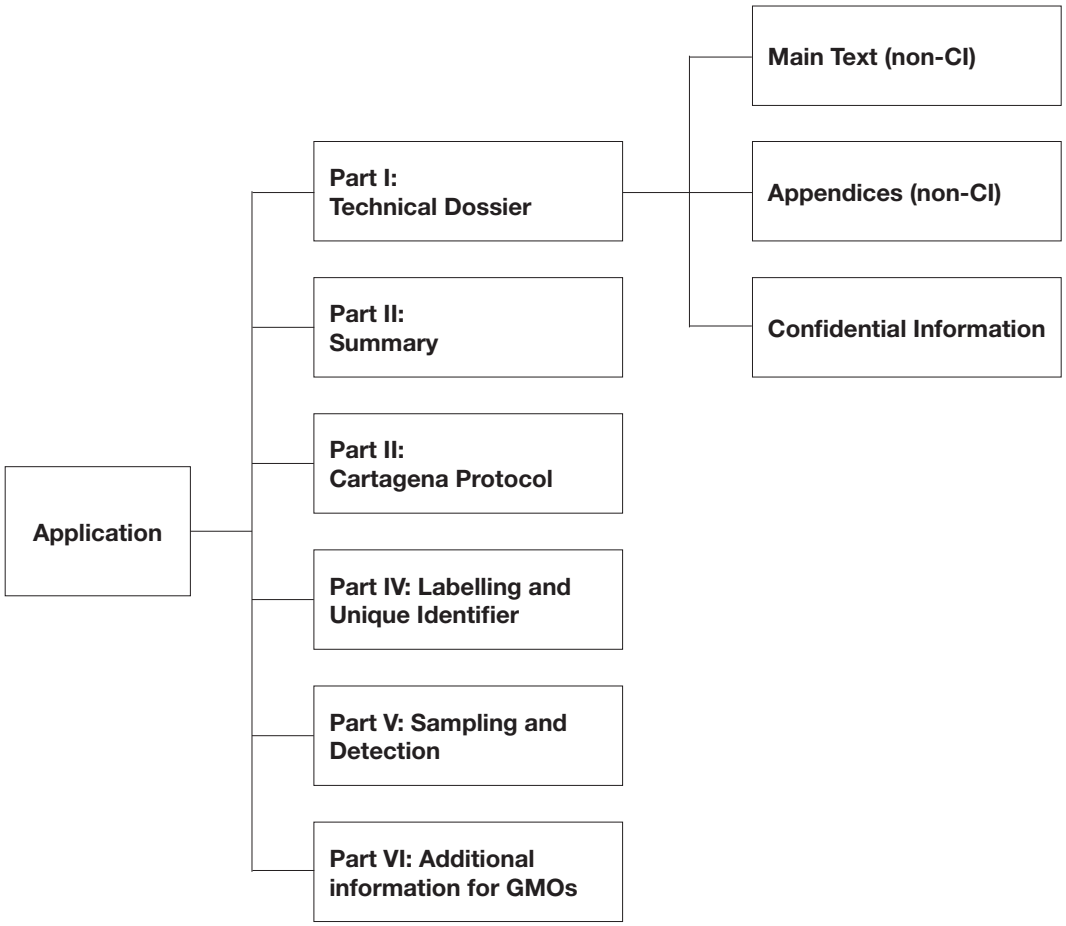
Application for the authorisation of GM Microorganisms and derived products intended for food and feed use

An application for the authorisation of a GMO and/or derived products submitted within the framework of Regulation (EC) 1829/2003 should preferably be presented in English and should consist of the particulars as specified by Articles 5 (3) and 17 (3) of that Regulation and as further detailed in Regulation (EC) 641/2004[12].

In the case of an application relating to a GMO for food or feed use, references to "food" or "feed" shall be interpreted as referring to food or feed containing, consisting of or produced from a GMO according to Articles 5 and 17 (4) of Regulation (EC) 1829/2003 in respect of which an application is made.

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

10 - *Regulation (EC) No 1829/2003 on genetically modified food and feed, OJ L 268, 18.10.2003, p. 1.*
11 - *Directive 2001/18/EC on the deliberate release into the environment of GMOs and repealing Council Directive 90/220/EEC, OJ L 106, 17.4.2001, p. 1*
12 - *Regulation (EC) No 641/2004 on detailed rules for the implementation of Regulation (EC) No 1829/2003 of the European Parliament and of the Council as regards the application for the authorisation of new genetically modified food and feed, the notification of existing products and adventitious of technically unavoidable presence of genetically modified material which has benefited from a favourable risk evaluation, OJ L 102, 7.4.2004, p. 14.*


Application
Part I:
Technical Dossier
Part II:
Summary
Part II:
Cartagena Protocol
Part IV: Labelling and
Unique Identifier
Part V: Sampling and
Detection
Part VI: Additional
information for GMOs
Main Text (non-CI)
Appendices (non-CI)
Confidential Information

PART I : Technical dossier

- The technical dossier should contain all necessary information for the risk assessment and should be structured according to the format of Annex III as proposed in the EFSA guidance document on GMMs and their derived products intended for food and feed use. Following Annex III and taking into account the detailed considerations from the Guidance document to each topic, the technical dossier should comprise the complete information required by Regulation (EC) 1829/2003 (Articles 5 and 17 (3) (a), (b), (d), (e), (h), (k). In the case of GMOs or food containing or consisting of GMOs, the technical dossier should also comprise the information required by Articles 5 and 17 (5) (a), (b). Applications submitted within the framework of Directive 2001/18/EC have to respect the technical requirements and formats set up by this Directive. Given the fact that such applications may lead to a consultation of the GMO Panel according to Article 28 of the Directive, the application should preferably also be compiled according to this EFSA guidance document.

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

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

  A copy of the studies as referred to in Articles 5 and 17 (3) (e) of Regulation (EC) 1829/2003 should be included as appendices to the main text of the technical dossier. A summary of the data and cross-references to these studies should be made in the main text. The application shall clearly state which parts of the application are considered to be confidential in accordance with Article 2 (3) of Regulation (EC) 641/2004, together with a verifiable justification in accordance with Article 30 of Regulation (EC) 1829/2003. Confidential information (CI) that is part of the technical dossier should be submitted as a separate file under Part I of the application.

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

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

  Not all the points included in the guidance document will apply to every case. In cases where a provision of the guidance document does not apply for a certain application, reasons must be given for the omission of such data from the dossier. It is to be expected that individual applications will address only the particular

subset of considerations that is appropriate to individual situations. The level of detail required in response to each subset of considerations is also likely to vary according to the scope of the application. The applicant should refer to Table 1 of this guidance in order to identify the requirements needed for the group which the GMM and/or derived product belongs to, according to the scope of the application as defined in Annex II.

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

PART II : Summary

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

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

PART III : Cartagena Protocol

Part III of the application shall apply only to applications concerning GMOs for food/feed use, or in the case of food/feed containing or consisting of GMOs. In these cases, Part III of the application should specify, in supplying the information required under Articles 5 and 17 (3) (c) of Regulation (EC) No 1829/2003, whether the information included in the application may be notified as such to the Biosafety Clearing-House under the Cartagena Protocol on Biosafety to the Convention on Biological Diversity (the Cartagena Protocol) approved by Council Decision 2002/628/EC[13].

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

PART IV : Labelling and unique identifier

Part IV of the application should comprise a proposal for labelling in accordance with Articles 12-14 and Articles 24-26 of Regulation (EC) 1829/2003. In the case of GMOs, food and/or feed containing or consisting of GMOs (Articles 5 and 17 (5)), a proposal for labelling has to be included complying with the requirements of Article 4, B (6) of Regulation (EC) 1830/2003 and Annex IV of Directive 2001/18/EC.

In supplying the information required under Articles 5 and 17 (5) (a) of Regulation (EC) 1829/2003, a proposal for a unique identifier for the GMO in question, developed in accordance with Commission Regulation (EC) 65/2004[14], should be given.

According to Article 3 (1) (d) of Regulation (EC) 641/2004, a proposal for labelling in all official Community languages should be provided, where a proposal for specific labelling is needed in accordance with Articles 5 and 17 (3) (f) (g) of Regulation (EC) 1829/2003.

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

14 - *Commission Regulation (EC) No 65/2004 of 14 January 2004 establishing a system for the development and assignment of unique identifiers for genetically modified organisms, OJ L 10, 16.1.2004, p. 5.*

PART V : Sampling and detection

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

Samples of the food or feed and their control samples which are to be submitted in accordance with Articles 5 and 17 (3) (j) of Regulation (EC) 1829/2003 should be in accordance with the requirements set out in Annexes I and II to Regulation (EC) 641/2004. The application should be accompanied by information concerning the place where the reference material developed in accordance with Annex II of Regulation (EC) 641/2004 can be accessed.

A format to provide information on GM detection methods and related samples can be found on the website of the Community Reference Laboratory (http://gmo-crl.jrc.it). For practical reasons, the methods for detection and sampling and the samples of the food and/or feed and control samples should be sent directly to the Joint Research Centre (JRC). A copy of the completed form, as found in Annex V of this guidance, and proof of sending to the JRC, should be provided in Part V of the application.

PART VI: Additional information for GMOs and/or food/feed containing or consisting of GMOs

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

Table with cross-references between the different parts of the application as specified by the Annexes of the guidance document and Regulation (EC) 1829/2003

Guidance document: specifications for the format of an application	Regulation (EC) 1829/2003
Part I: Technical Dossier	Articles 5&17 (3) (a) (b) (d) (e) (h) (k); Articles 5&17 (5) (a) (b)
Part II: Summary	Articles 5&17 (3) (l)
Part III: Cartagena Protocol	Articles 5&17 (3) (c)
Part IV: Labelling	Articles 5&17 (3) (f) (g); Articles 5&17 (5) (a); Articles 12-14 and Articles 24-26
Part V: Sampling and Detection	Articles 5&17 (3) (i) (j)
Part VI: Additional information for GMOs and/or food/feed containing or consisting of GMOs	Articles 5&17 (5), more specifically, Annex IV of Directive 2001/18/EC

Practical specifications

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

European Food Safety Authority
Scientific Coordinator GMO Panel
Address:
Largo N. Palli 5/A, I-43100 Parma
I-43100 Parma
Italy

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

EFSA has to make the application available to the Member States and to the Commission as required by Articles 5 and 17 (2) (b) of Regulation (EC) 1829/2003. For this purpose, EFSA will use a secure electronic system (GMO EFSAnet) to make the electronic version of applications available to them.

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

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

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

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

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

Annex II

Scope of the application

The scope of the application should be defined very clearly. It should be indicated whether the GMM and/or its derived products are intended for food use, for feed use, or for both food and feed use and whether or not the GMM will be deliberately released into the environment.

It should also be indicated which of the following group(s)[15] the product(s) belongs to:

Group 1: Single compounds or defined mixtures of compounds derived from GMMs

Group 2: Complex products derived from GMMs but not containing viable GMMs nor unit length of any cloned (foreign) open reading frames

Group 3: GMMs and products containing viable GMMs or genetically intact cloned (foreign) DNA

Where the application is limited to either food or feed use, it shall contain a verifiable justification explaining why the authorisation should not cover both uses in accordance with Article 27 of Regulation (EC) 1829/2003.

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

15 - Groups defined in Chapter II.2. of this guidance.

Annex III

Format of technical dossiers

Information required in applications for GM microorganisms and/or derived products intended for food and feed use

A. GENERAL INFORMATION

1. Name and address of the applicant (company or institute)

2. Name, qualification and experience of the responsible scientist(s) and contact details of the responsible person for all dealings with EFSA

3. Title of the project

4. Scope of the application as defined in Annex II

5. Designation and specification of the GM microorganism and/or derived product, including its proprietary name, the generic and commercial names of the product, production strain, etc.

6. Where applicable, a detailed description of the method of production and manufacturing

7. Where appropriate, 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

B. INFORMATION RELATING TO THE GMM

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

The applicant should provide a comprehensive description of the recipient microorganism or the parental strain in the case of a microorganism in which the endogenous genetic material has been modified. Its history of safe use should be described. In cases in which microorganisms that contain virulence determinants are used as recipients or parental organisms, their use must be justified in the application. In case of a parental or recipient microorganism with the status of QPS for the equivalent end use, the information requirements will be reduced (see Table 1). Information relating to the recipient or (when appropriate) the parental organism must include the following:

1.1. Identity: (a) common name, (b) strain designation, (c) source of the strain, (d) accession number from a recognised culture collection, if available

1.2. Taxonomy: (a) genus, (b) species, (c) subspecies (if appropriate), (d) strain

1.3. Other names: (when appropriate) (a) generic name, (b) commercial name, (c) previous name(s)

1.4. Phenotypic and genetic markers: (a) phenotypic and genotypic information rele vant to identification, genetic stability and safety, (b) information on pathogenicity

1.5. Degree of relatedness between recipient and donor(s), when appropriate

1.6. Description of identification and detection techniques

1.7. Sensitivity, reliability and specificity of the detection techniques

1.8. Source and natural habitat of the recipient microorganism

1.9. Organisms with which transfer of genetic material is known to occur under natural conditions

1.10. Information on the genetic stability of the recipient microorganism

1.11. Pathogenicity, ecological and physiological traits

(a) classification of hazard according to the current Community legislation

(b) information on the doubling time and of the mode of reproduction

(c) information on survival, ability to form spores or other survival structures

(d) infectivity

(e) toxigenicity

(f) virulence

(g) allergenicity

(h) information on viability and ability to survive in the gastrointestinal tract of humans or animals

(i) probiotic or immunomodulatory properties

(l) presence of genes that confer antibiotic resistance

(m) involvement in environmental processes

1.12. Information on indigenous mobile genetic elements

1.13. Description of its history of use

1.14. History of previous genetic modifications

2. Characteristics of the donor organism(s)

2.1. Identity: (a) common name, (b) strain designation, (c) source of the strain, (d) accession number from a recognised culture collection, if available

2.2. Taxonomy: (a) genus, (b) species, (c) subspecies (if appropriate), (d) strain

2.3. Other names: (a) generic name, (b) commercial name, (c) previous name(s)

2.4. Phenotypic and genetic markers: (a) phenotypic and genotypic information relevant to identification, genetic stability and safety, (b) information on pathogenicity

2.5. Description of identification and detection techniques

2.6. Sensitivity, reliability and specificity of the detection techniques

2.7. Source and habitat of the organism

2.8. Pathogenicity traits: (a) classification of hazard according to the current Community legislation, (b) pathogenicity, (c) infectivity, (d) toxigenicity, (e) virulence, (f) allergenicity, (g) ability to act as carrier of pathogenicity islands

2.9. History of use

3. Description of the genetic modification process

3.1 Characteristics of the vector

(a) nature and source of the vector

(b) the copy number

(c) physical and genetic map

(d) position and nucleotide sequence of probes and primers used

(e) identification and description of each component

(f) frequency of mobilisation of the vector and its capacity for genetic transfer

(g) information relating to the host range of plasmid used as a vector

3.2 Information relating to the genetic modification

(a) methods used to construct and introduce the insert(s) into the recipient or to delete a sequence(s)

(b) integration site, sequence actually inserted or deleted, size and copy number of all detectable inserts

(c) methods used for their detection

(d) size and function of the deleted region(s)

(e) purity of the insert

(f) sequence of flanking regions

(g) methods and criteria used for selection

(h) subcellular location(s) of insert(s)

4. Identification of the conventional counterpart microorganism and its characteristics

Description of all relevant phenotypic and genotypic traits of the comparator: (a) methods used to establish the identity of the comparator, (b) comparative risk assessment of the most relevant key components (metabolic activity, physiology, safety, etc.), (c) presence of mobile genetic elements (plasmids, transposons, integrons and prophage), (d) genetic stability and variability

5. Information relating to the GMM and comparison of the GMM with its conventional counterpart

5.1 Description of the genetic trait(s) or phenotypic characteristics and any new trait which can be expressed or no longer expressed: identification and description of any qualitative and quantitative difference between the GMM and its comparator

5.2 Structure and amount of any vector and/or donor nucleic acid remaining in the final construction of the modified microorganism

5.3 Stability of the microorganism in terms of genetic traits

5.4 Rate and level of expression of the new genetic material

5.5 Description of identification and detection techniques

5.6 Information on the ability to transfer genetic material to other organisms

5.7 Information on the interaction of the GMM with other organisms

5.8 History of previous releases or uses of the GMM

5.9 Safety for humans and animals

(a) information on any toxic, allergenic or other harmful effects on human or animal health

(b) potential for DNA transfer or any capacity for enhanced gene transfer

(c) viability and residence time of the GMM in the alimentary tract

(d) information on any impact of the GMM on the microbiota of the human or animal gastrointestinal tract

5.10 Information on monitoring, control, waste treatment and emergency response plans

C. INFORMATION RELATING TO THE GM PRODUCT

1. Information relating to the production process

2. Information relating to the product purification process

2.1 Technique used to remove microbial cells from the product

2.2 Information on the technique used to kill the microbial cells

2.3 Information on the process used to purify the product from the microbial growth medium

3. Description of the product

3.1 Designation of the product

3.2 Intended use and mode of action

3.3 Composition

3.4 Physical properties

3.5 Technological properties

4. Assessment of the presence of recombinant DNA and of the potential risk of gene transfer

5. Comparison of the GM product with its conventional counterpart

6. Considerations for human health and animal health of the GM product

6.1 Toxicology

6.2 Risk assessment of newly expressed proteins

6.3 Testing of new constituents other than proteins

6.4 Information on natural food and feed constituents

6.5 Testing of the whole GM product

6.6 Allergenicity

6.7 Assessment of allergenicity of newly expressed protein

6.8 Assessment of allergenicity of the whole GM product

6.9 Nutritional assessment

6.9.1 Nutritional assessment of the GM food

6.9.2 Nutritional assessment of the GM feed

6.10 Post-market monitoring of GM products

D. POTENTIAL ENVIRONMENTAL IMPACT OF GMMs AND DERIVED PRODUCTS

1. Environmental Assessment for Level 1 cases

1.1 Spread of the GMM from the product to external environments

1.2 General ability of the GMM to survive and persist in external environments

1.3 Transfer of recombinant DNA

2. Environmental Assessment for Level 2 cases

2.1 The potential for survival in receiving environments and selective advantage

2.2 The potential for transfer of recombinant genes

2.3 Effects on indigenous microorganisms

2.4 Effects on humans

2.5 Effects on animals

2.6 Effects on plants

2.7 Effects on biogeochemical processes

3. Environmental monitoring plan

3.1 General

3.2 Interplay between environmental risk assessment and monitoring

3.2.1 Monitoring of effects: foreseen and unforeseen

3.2.2 Monitoring framework

3.3 Case-specific GM monitoring

3.4 General surveillance of the impact of the GMM

3.4.1 Approach and principles

3.4.2 Main elements of General Surveillance

3.5 Monitoring systems

3.6 Reporting the results of monitoring

Annex IV

Format[16] of the summary of applications for genetically modified microorganisms and/or derived products intended for food and feed use

According to Articles 5(3)(l) and 17(3)(l) of Regulation (EC) 1829/2003, the application shall be accompanied by a summary of the dossier in a standardised form. This annex specifies the format of such summary for genetically modified microorganisms and/or derived products intended for food and feed use. Depending on the scope of the application, some of the specifications may not be applicable. The summary shall be presented in an easily comprehensible and legible form. It shall not contain parts which are considered to be confidential.

A. GENERAL INFORMATION

1. Details of application

a) Member State of application
b) Application number
c) Name of the product (commercial and other names)
d) Date of acknowledgement of valid application

2. Applicant

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

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

3. Scope of the application

☐ GM microorganisms and/or derived products for food use

☐ GM microorganisms and/or derived products for feed use

☐ GM microorganisms and/or derived product(s) belonging to Group 1, as defined in Chapter II, 2. of this guidance

☐ GM microorganisms and/or derived product(s) belonging to Group 2, as defined in Chapter II, 2. of this guidance

☐ GM microorganisms and/or derived product(s) belonging to Group 3, as defined in Chapter II, 2. of this guidance

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

4. Is the product being simultaneously notified within the framework of another regulation?

Yes ☐	No ☐
If yes, specify	

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

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

6. Has the GM microorganism or derived products been previously notified for marketing in the Community under Part C of Directive 2001/18/EC or Regulation (EC) 258/97?

Yes ☐	No ☐
If yes, specify	

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

Yes ☐	No ☐

8. General description of the product

a) Name of the recipient or parental microorganism and the intended function of the genetic modification
b) Types of products planned to be placed on the market according to the authorisation applied for
c) Intended use of the product and types of users
d) Specific instructions and/or recommendations for use, storage and handling, including mandatory restrictions proposed as a condition of the authorisation applied for
e) Any proposed packaging requirements
f) A proposal for labelling in accordance with Articles 13 and Articles 25 of Regulation (EC) 1829/2003. In the case of GMOs, food and/or feed containing or consisting of GMOs, a proposal for labelling has to be included complying with the requirements of Article 4, B(6) of Regulation (EC) 1830/2003 and Annex IV of Directive 2001/18/EC
g) Unique identifier for the GM microorganism in accordance with Regulation (EC) 65/2004
h) If applicable, geographical areas within the EU to which the product is intended to be confined under the terms of the authorisation applied for. Any type of environment to which the product is unsuited

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

B. INFORMATION RELATING TO THE GMM

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

1.1 Identity

a) Common name
b) Strain designation
c) Source of the strain
d) Accession number from a recognised culture collection

1.2 Taxonomy

a) Genus
b) Species
c) Subspecies
d) Strain

1.3 Other names

a) Generic name
b) Commercial name
c) Previous name(s)

1.4 Phenotypic and genetic markers

a) Phenotypic and genotypic information relevant to identification, genetic stability and safety
b) Information on pathogenicity

1.5 Degree of relatedness between recipient and donor(s), when appropriate

1.6 Description of identification and detection techniques

1.7 Sensitivity, reliability and specificity of the detection techniques

1.8 Source and natural habitat of the recipient microorganism

1.9 Organisms with which transfer of genetic material is known to occur under natural conditions

1.10 Information on the genetic stability of the recipient microorganism

1.11 Pathogenicity, ecological and physiological traits

a) Classification of hazard according to the current Community legislation
b) Information on the doubling time and of the mode of reproduction
c) Information on survival, ability to form spores or other survival structures
d) Infectivity
e) Toxigenicity
f) Virulence
g) Allergenicity
h) Information on viability and ability to survive in the gastrointestinal tract of humans or animals
i) Probiotic or immunomodulatory properties
l) Presence of genes that confer antibiotic resistance
m) Involvement in environmental processes

1.12 Information on indigenous mobile genetic elements

1.13 Description of its history of use

1.14 History of previous genetic modifications

2. Characteristics of the donor organism(s)

2.1 Identity

a) Common name
b) Strain designation
c) Source of the strain
d) Accession number from a recognised culture collection

2.2 Taxonomy

a) Genus
b) Species
c) Subspecies
d) Strain

2.3 Other names

a) Generic name
b) Commercial name
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2.4 Phenotypic and genetic markers

a) Phenotypic and genotypic information relevant to identification, genetic stability and safety
b) Information on pathogenicity

2.5 Description of identification and detection techniques

2.6 Sensitivity, reliability and specificity of the detection techniques

2.7 Source and habitat of the organism

2.8 Pathogenicity traits

a) Classification of hazard according to the current Community legislation
b) Pathogenicity
c) Infectivity
d) Toxigenicity
e) Virulence
f) Allergenicity
g) Ability to act as carrier of pathogenicity islands

2.9 Description of its history of use

3. Description of the genetic modification process

3.1 Characteristics of the vector

a) Nature and source of the vector
b) The copy number
c) Physical and genetic map
d) Position of probes and primers used
e) Identification and description of each component
f) Frequency of mobilisation of the vector and its capacity for genetic transfer
g) Information relating to the host range of plasmid used as a vector

3.2 Information relating to the genetic modification

a) Methods used to construct and introduce the insert(s) into the recipient or to delete a sequence(s)
b) Integration site, sequence actually inserted or deleted, size and copy number of all detectable inserts
c) Methods used for their detection
d) Size and function of the deleted region(s)
e) Purity of the insert
f) Sequence of flanking regions
g) Methods and criteria used for selection
h) Subcellular location(s) of insert(s)

4. Identification of the conventional counterpart microorganism and its characteristics

5. Information relating to the GMM and comparison of the GMM with its conventional counterpart

5.1 Description of the genetic trait(s) or phenotypic characteristics and any new trait which can be expressed or no longer expressed

5.2 Structure and amount of any vector and/or donor nucleic acid remaining in the final construction of the modified microorganism

5.3 Stability of the microorganism in terms of genetic traits

5.4 Rate and level of expression of the new genetic material

5.5 Description of identification and detection techniques

5.6 Information on the ability to transfer genetic material to other organisms

5.7 Information on the interaction of the GMM with other organisms

5.8 History of previous releases or uses of the GMM

5.9 Safety for humans and animals

a) Information on any toxic, allergenic or other harmful effects on human or animal health
b) Potential for DNA transfer or any capacity for enhanced gene transfer
c) Viability and residence time of the GMM in the alimentary tract
d) Information on any impact of the GMM on the microbiota of the human or animal gastrointestinal tract

5.10 Information on monitoring, control, waste treatment and emergency response plans

C. INFORMATION RELATING TO THE GM PRODUCT

1. Information relating to the production process

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2.2 Information on the technique used to kill the microbial cells

2.3 Information on the process used to purify the product from the microbial growth medium

3. Description of the product

3.1 Designation of the product

3.2 Intended use and mode of action

3.3 Composition

3.4 Physical properties

3.5 Technological properties

4. Assessment of the presence of recombinant DNA and of the potential risk of gene transfer

5. Comparison of the GM product with its conventional counterpart

6. Considerations for human health and animal health of the GM product

6.1 Toxicology

6.2 Risk assessment of newly expressed proteins

6.3 Testing of new constituents other than proteins

6.4 Information on natural food and feed constituents

6.5 Testing of the whole GM product

6.6 Allergenicity

6.7 Assessment of allergenicity of newly expressed protein

6.8 Assessment of allergenicity of the whole GM product

6.9 Nutritional assessment

6.9.1 Nutritional assessment of GM food

6.9.2 Nutritional assessment of GM feed

6.10 Post-market monitoring of GM products

D. POTENTIAL ENVIRONMENTAL IMPACT OF GMMs AND DERIVED PRODUCTS

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1.2 General ability of the GMM to survive and persist in external environments

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2.2 The potential for transfer of recombinant genes

2.3 Effects on indigenous microorganisms

2.4 Effects on humans

2.5 Effects on animals

2.6 Effects on plants

2.7 Effects on biogeochemical processes

3. Environmental monitoring plan

3.1 General

3.2 Interplay between environmental risk assessment and monitoring

3.2.1 Monitoring of effects: foreseen and unforeseen
3.2.2 Monitoring framework

3.3 Case specific GM monitoring

3.4 General surveillance of the impact of the GMM

3.4.1 Approach and principles
3.4.2 Main elements of General Surveillance

3.5 Monitoring systems

3.6 Reporting the results of monitoring

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

1. History of previous releases of the GM microorganism notified under Part B of the Directive 2001/18/EC and under Part B of Directive 90/220/EEC by the same notifier

a) Notification number
b) Conclusions of post-release monitoring
c) Results of the release in respect to any risk to human health and the environment (submitted to the Competent Authority according to Article 10 of Directive 2001/18/EC)

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

a) Release country
b) Authority overseeing the release
c) Release site
d) Aim of the release
e) Duration of the release
f) Aim of post-releases monitoring

g) Duration of post-releases monitoring
h) Conclusions of post-release monitoring
i) Results of the release in respect to any risk to human health and the environment

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

a) Status/process of approval
b) Assessment Report of the Competent Authority (Directive 2001/18/EC)
c) EFSA opinion
d) Commission Register (Commission Decision 2004/204/EC[17])
e) Molecular Register of the Community Reference Laboratory/Joint Research Centre
f) Biosafety Clearing-House (Council Decision 2002/628/EC[18])
g) Summary Notification Information Format (SNIF) (Council Decision 2002/812/EC)

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

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

Annex V

Submission of samples to the European Commission - DG Joint Research Centre

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

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

Reference: Date:

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

1. Name of the food and/or feed:
2. Trade name (where applicable):
3. Transformation event:
4. Unique identifier as defined in Regulation (EC) 65/2004 (only applicable for GMOs):
5. Place where the reference material can be assessed:

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

EFSA: GMO@efsa.europa.eu

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

Yours faithfully,

Signature:

Enclosures: samples, control samples

INSTRUCTIONS AND INFORMATION

- The preparation of the samples and control samples shall follow the specifications laid down in: http://gmo-crl.jrc.it
- The parcel shall be specified to contain “Free samples”, and it shall include the list of all items and their storage instructions. In addition, it is recommended to send an advance notice of the arriving delivery (*e.g.* at the time of shipment) to: gmo-validation@jrc.it
- A copy of this letter should be included in Part V of the application as specified in Annex I of the EFSA Guidance on GM Microorganisms and their derived products intended for food and feed use
- Regulation (EC) No 1829/2003 on genetically modified food and feed (OJ L 268, 18.10.2003, p. 1)
- Regulation (EC) No 641/2004 on detailed rules for the implementation of Regulation (EC) No 1829/2003 (OJ L 102, 7.4.2004, p. 14)
- http://www.efsa.europa.eu
- http://ec.europa.eu/food/index_en.htm

Acknowledgement of receipt

Submission of samples of the food/feed and their control samples referred to in Articles 5(3) (j) and 17(3)(j) of Regulation (EC) 1829/2003 for applications for authorisations in accordance with Articles 5 and 17 of that Regulation and Article 4(1) and Annexes I and II of Regulation (EC) 641/2004

Please write your return address below:

Reference:

I confirm that the samples and control samples, concerning the product as specified below have been received by the European Commission, Directorate-General Joint Research Centre, and will be the subject of the verification provided by Article 5 and/or 17 of Regulation (EC) 1829/2003.

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

Name of the food and/or feed:

Trade name (where applicable):

Short description:

Date: (dd/mm/yyyy)

Signature:
Guy Van den Eede, Head of Unit

Stamp:

Annex VI

Correlation table comparing the required information according to Regulation (EC) 1829/2003 and the Guidance Document (GD)

If the product contains or consists of GMO, specific information has to be included as stipulated under Art. 5 of Regulation (EC) 1829/2003 (no shading) referring to annexes II, III, IV, and VII of Directive 2001/18/EC (blue shading). For feed (Art. 17) the same correlation system is valid. Differences between the GD and the legal requirements are underlined.

	Text Regulation or Directive	GD Annex/chapter	Correlating parts in Annexes of the Guidance Document	Dossier
1829/2003				
Art. 5(3)				
(a)	the name and the address of the applicant;	Annex III/A.1	Name and address of the applicant (company or institute	Part I
(b)	the designation of the food, and its specification, including the transformation event(s) used;	Annex III/A.5	Designation and specification of the GM microorganism and/or derived product, including its proprietary name, the generic and commercial names of the product, production strain, etc.	Part I
(c)	where applicable, the information to be provided for the purpose of complying with Annex II to the Cartagena Protocol on Biosafety to the Convention on Biological Diversity (hereinafter referred to as the Cartagena Protocol);;	Annex I	see Annex I, Part III	Part III
(d)	where applicable, a detailed description of the method of production and manufacturing	Annex III/A.6	Where applicable, a detailed description of the method of production and manufacturing	Part I

	Text Regulation or Directive	GD Annex/chapter	Correlating parts in Annexes of the Guidance Document	Dossier
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);	Annex I in general	remark: Annex III A from Directive 2001/18/EC was the starting point for GD and respective Annexes	Part I
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);	Annex I	see Annex I, Part IV	Part IV
g)	either a reasoned statement that the food does not give rise to ethical or religious concerns, or a proposal for labelling it in accordance with Article 13(2)(b);	Annex I	see Annex I, Part IV	Part IV
h)	where appropriate, the conditions for placing on the market the food or foods produced from it, including specific conditions for use and handling;	Annex III/A.7	Where appropriate, 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	Part I
i)	methods for detection, sampling (including references to existing official or standardised sampling methods) and identification of the transformation event and, where applicable; for the detection and identification of thtransformation event in the food and/or in foods produced from it;	Annex I	see Annex I, Part V	Part V

	Text Regulation or Directive	GD Annex/chapter	Correlating parts in Annexes of the Guidance Document	Dossier
j)	samples of the food and their control samples, and information as to the place where the reference material can be accessed;	Annex I	see Annex I, Part V	Part V
k	where appropriate, a proposal for post-market monitoring regarding use of the food for human consumption;	Annex III/ C.6.10	Post-market monitoring of GM products	Part I
l)	a summary of the dossier in a standardised form;	Annex I	see Annex I, Part II	Part II
Art. 5(5)	**Food/feed containing or consisting of GMO**			
a)	Reference to Annexes II, IIIA, and IV of 2001/18/ EC or where the GMO is already authorised under part C of the Directive → copy of authorisation decision			
b)	Monitoring plan according to Annex VII of 2001/18			
2001/18				
Annex II/D.1	Conclusions of the potential environmental impact from the release or the placing on the market of GMOs	Annex III/D	**Potential environmental impact of GMMs and derived products**	Part I
Annex III/A		Annex III		
	I. GENERAL INFORMATION		**A. GENERAL INFORMATION**	
A.1	Name and address of the notifier (company or institute	Annex III/A.1	Name and address of the applicant (company or institute)	Part I

	Text Regulation or Directive	GD Annex/chapter	Correlating parts in Annexes of the Guidance Document	Dossier
A.2	Name, qualifications and experience of the responsible scientist(s)	Annex III/A.2	Name, qualification and experience of the responsible scientist(s) and contact details of the responsible person for all dealings with EFSA	Part I
A.3	Title of the project	Annex III/A.3	Title of the project	Part I
	II. INFORMATION RELATING TO THE GMO		**B. INFORMATION RELATING TO THE GMO**	
	A. Characteristics of the (a) donor	Annex III/B.2	**Characteristics of the donor organism(s)**	Part I
A.a		Annex III/B.2		Part I
	1. scientific name		2.1 Identity	
	2. taxonomy		2.2 taxonomy	
	3. other names		2.3 other names	
	4. phenotypic and genetic markers		2.4 phenotypic and genetic markers	
	5. degree of relatedness between donor and recipient or between parental organisms			
	6. description of identification and detection techniques		2.5 description of identification and detection techniques	
	7. sensitivity, reliability and specificity of detection and identification techniques		2.6 sensitivity, reliability and specificity of detection techniques	
	8. description of the geographic distribution and of the habitat of the organism		2.7 source and natural habitat of the organism	

	Text Regulation or Directive	GD Annex/chapter	Correlating parts in Annexes of the Guidance Document	Dossier
	9. organisms with which transfer of genetic material is known to occur under natural conditions			
	10. verification of the genetic stability of the organisms and factors affecting it			
	11. pathological, ecological and physiological traits		2.8 Pathogenicity traits	
	12. nature of indigenous vectors			
	13. history of previous genetic modifications			
			2.9 History of use	
	A. Characteristics of (b) the recipient or (where appropriate) parental organism(s)		**Characteristics of (b) the recipient or (where appropriate) parental organism(s)**	Part I
A. b		Annex III/B.1		Part I
	1. scientific name		1.1 identity	
	2. taxonomy		1.2 taxonomy	
	3. other names		1.3 other names	
	4. phenotypic and genetic markers		1.4 phenotypic and genetic markers	
	5. degree of relatedness between donor and recipient or between parental organisms		1.5 degree of relatedness between recipient and donor(s), where appropriate	
	6. description of identification and detection techniques		1.6 description of identification and detection techniques	

	Text Regulation or Directive	GD Annex/chapter	Correlating parts in Annexes of the Guidance Document	Dossier
	7. sensitivity, reliability and specificity of detection and identification techniques		1.7 sensitivity, reliability and specificity of detection techniques	
	8. description of the geographic distribution and of the natural habitat of the organism		1.8 source and natural habitat of the recipient microorganism	
	9. organisms with which transfer of genetic material is known to occur under natural conditions		1.9 organisms with which transfer of genetic material is known to occur under natural conditions	
	10. verification of the genetic stability of the organisms and factors affecting		1.10 Information on the genetic stability of the recipient microorganism	
	11. pathological, ecological and physiological traits		1.11 Pathogenicity, ecological and physiological traits	
	12. nature of indigenous vectors		1.12 Information on indigenous mobile genetic elements	
	13. history of previous genetic modifications		1.14 history of previous genetic modifications	
			1.13 Description of its history of use	
		Annex III/B.3	**Description of the genetic modification process**	Part I

	Text Regulation or Directive	GD Annex/chapter	Correlating parts in Annexes of the Guidance Document	Dossier
	B. Characteristics of the vector		3.1 Characteristics of the vector	
B.1	8. Nature and source of the vector		(a) nature and source of the vector	
B.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		(e) identification and description of each component	
B.3	Frequency of mobilisation of inserted vector and/or genetic transfer capabilities and methods of determination		(f) frequency of mobilisation of the vector and its capacity for genetic transfer	
B.4	Information on the degree to which the vector is limited to the DNA required to perform the intended function		(e) identification and description of each component	
			(b) the copy number	
			(c) physical and genetic map	
			(d) position of probes and primers used	
			(g) information relating to the host range of plasmid used as a vector	

	Text Regulation or Directive	GD Annex/chapter	Correlating parts in Annexes of the Guidance Document	Dossier
	C. Characteristics of the modified organism			
C.1	**Information relating to the genetic modification**		3.2 Information relating to the genetic modification	Part I
	(a) methods used for the genetic modification			
	(b) methods used to construct and introduce the insert(s) into the recipient or to delete a sequence(s)		(a) methods used to construct and introduce the insert(s) into the recipient or to delete a sequence(s)	
	(c) description of the insert and/or vector construction		(b) integration site, sequence actually inserted or deleted, size and copy number of all detectable inserts (c) methods used for their detection	
	(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		(e) purity of the insert	
	(e) methods and criteria used for selection		(g) methods and criteria used for selection	

	Text Regulation or Directive	GD Annex/chapter	Correlating parts in Annexes of the Guidance Document	Dossier
	(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		(d) size and function of the deleted region(s) (f) sequence of flanking regions (h) the subcellular location(s) of insert(s)	
C.2	**Information on the final GMO**	Annex III/B.4 Annex III/B.5	**Identification of the conventional counterpart microorganism and its characteristics** **Information relating to the GMM and comparison of the GMM with its conventional counterpart**	Part I
	(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		5.1 Description of the genetic trait(s) or phenotypic characteristics and any new trait which can be expressed or no longer expressed: identification and description of any qualitative and quantitative difference between the GMM and its comparator	
	(b) Structure and amount of any vector and/or donor nucleic acid remaining in the final construction of the modified organism		5.2 Structure and amount of any vector and/or donor nucleic acid remaining in the final construction of the modified microorganism	

	Text Regulation or Directive	GD Annex/chapter	Correlating parts in Annexes of the Guidance Document	Dossier
	(c) Stability of the organism in terms of genetic traits		5.3 Stability of the microorganism in terms of genetic traits	
	(d) Rate and level of expression of the new genetic material. Method and sensitivity of measurement		5.4 Rate and level of expression of the new genetic material	
	(e) Activity of the expressed protein(s)		Addressed under Annex III/C.6.2	
	(f) Description of identification and detection techniques including techniques for the identification and detection of the inserted sequence and vector		5.5 Description of identification and detection techniques	
	(g) Sensitivity, reliability and specificity of detection and identification techniques		5.5 Description of identification and detection techniques	
	(h) History of previous releases or uses of the GMO		5.8 History of previous releases or uses of the GMM	
	(i) Considerations for human health and animal health as well as plant health		5.9 Safety for humans and animals	
			5.6 Information on the ability to transfer genetic material to other organisms	

	Text Regulation or Directive	GD Annex/chapter	Correlating parts in Annexes of the Guidance Document	Dossier
			5.7 Information on the interaction of the GMM with other organisms	
			5.10 Information on monitoring, control, waste treatment and emergency response plans	
		Annex III/C	**C. INFORMATION RELATING TO THE GM PRODUCT**	Part I
		Annex III/C.1	**Information relating to the production process**	Part I
		Annex III/C.2	**Information relating to the product purification process**	Part I
			2.1 Technique used to remove microbial cells from the product	
			2.2 Information on the technique used to kill the microbial cells	
			2.3 Information on the process used to purify the product from the microbial growth medium	

	Text Regulation or Directive	GD Annex/chapter	Correlating parts in Annexes of the Guidance Document	Dossier
		Annex III/C.3	**Description of the product**	Part I
			3.1 Designation of the product	
			3.2 Intended use and mode of action	
			3.3 Composition	
			3.4 Physical properties	
			3.5 Technological properties	
		Annex III/C.4	**Assessment of the presence of recombinant DNA and the potential risk of gene transfer**	Part I
		Annex III/C.5	**Comparison of the GM product with its conventional counterpart**	Part I
		Annex III/C.6	**Considerations for human health and animal health of the GM product**	Part I
			6.1 Toxicology	
			6.2 Risk assessment of newly expressed proteins	

	Text Regulation or Directive	GD Annex/chapter	Correlating parts in Annexes of the Guidance Document	Dossier
			6.3 Testing of new constituents other than proteins	
			6.4 Information on natural food and feed constituents	
			6.5 Testing of the whole GM product	
			6.6 Allergenicity	
			6.7 Assessment of allergenicity of newly expressed protein(s)	
			6.8 Assessment of allergenicity of the whole GM product	
			6.9 Nutritional assessment	
			6.10 Post market monitoring of GM products	
Annex III/A	**III. INFORMATION RELATING TO THE CONDITIONS OF RELEASE AND THE RECEIVING ENVIRONMENT**	Annex III/D Annex III/D.1	**POTENTIAL ENVIRONMENTAL IMPACT OF GMMs AND DERIVED PRODUCTS**	Part I Part I
	IV. INFORMATION RELATING TO THE INTERACTIONS BETWEEN THE GMOs AND THE ENVIRONMENT	Annex III/D.2	Environmental Assessment for Level 1 cases Environmental Assessment for Level 2 cases	Part I

	Text Regulation or Directive	GD Annex/chapter	Correlating parts in Annexes of the Guidance Document	Dossier
Annex III/A	**V. INFORMATION ON MONITORING, CONTROL, WASTE TREATMENT AND EMERGENCY RESPONSE PLANS**	Annex III/D.3	Environmental monitoring plan	Part I
Annex IV	**Additional Information**	Annex I	see Annex I, Part VI	Part VI
Annex VII	**MONITORING PLAN** This Annex describes in general terms the objective to be achieved and the general principles to be followed to design the monitoring plan referred to in Articles 13(2), 19(3) and 20. It will be supplemented by guidance notes to be developed in accordance with the procedure laid down in Article 30(2). See also COUNCIL DECISION of 3 October 2002 (2002/811/EC)		see Annex I, Part I	Part I

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