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Foods derived from modern biotechnology

Second edition

World Health Organization

Food and Agriculture Organization of the United Nations

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WORLD HEALTH ORGANIZATION

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THE CODEX ALIMENTARIUS COMMISSION

The Codex Alimentarius Commission is an intergovernmental body with more than 180 members, within the framework of the Joint Food Standards Programme established by the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO), with the purpose of protecting the health of consumers and ensuring fair practices in the food trade. The Commission also promotes coordination of all food standards work undertaken by international governmental and non-governmental organizations.

The *Codex Alimentarius* (Latin, meaning Food Law or Code) is the result of the Commission's work: a collection of internationally adopted food standards, guidelines, codes of practice and other recommendations. The texts in this publication are part of the Codex Alimentarius.

FOODS DERIVED FROM MODERN BIOTECHNOLOGY
Second edition

The texts in this publication represent the outcome of the work of the Codex Alimentarius Commission on principles and guidelines for food safety assessment of foods derived from modern biotechnology. They give guidance on how to assess the safety of such foods and thus protect the health of consumers. This second edition includes texts adopted by the Codex Alimentarius Commission up to 2008.

Further information on these texts, or any other aspect of the Codex Alimentarius Commission, may be obtained from:

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PRINCIPLES FOR THE RISK ANALYSIS OF FOODS DERIVED FROM MODERN BIOTECHNOLOGY

CAC/GL 44-2003

SECTION 1 – INTRODUCTION

1. For many foods, the level of food safety generally accepted by society reflects the history of their safe consumption by humans. It is recognized that in many cases the knowledge required to manage the risks associated with foods has been acquired in the course of their long history of use. Foods are generally considered safe provided that care is taken during development, primary production, processing, storage, handling and preparation.

2. The hazards associated with foods are subjected to the risk analysis process of the Codex Alimentarius Commission to assess potential risks and, if necessary, to develop approaches to manage these risks. The conduct of risk analysis is guided by general decisions of the Codex Alimentarius Commission[1] as well as the *Working Principles for risk analysis*.[2]

3. While risk analysis has been used over a long period of time to address chemical hazards (e.g. residues of pesticides, contaminants, food additives and processing aids), and it is being increasingly used to address microbiological hazards and nutritional factors, the principles were not elaborated specifically for whole foods.

4. The risk analysis approach can, in general terms, be applied to foods including foods derived from modern biotechnology. However, it is recognized that this approach must be modified when applied to a whole food rather than to a discrete hazard that may be present in food.

5. The principles presented in this document should be read in conjunction with the *Working Principles for risk analysis* to which these principles are supplemental.

6. Where appropriate, the results of a risk assessment undertaken by other regulatory authorities may be used to assist in the risk analysis and avoid duplication of work.

[1] These decisions include the "Statements of principle concerning the role of science in the Codex decision-making process and the extent to which other factors are taken into account" and the "Statements of principle relating to the role of food safety risk assessment" (*Codex Alimentarius Commission Procedural Manual,* 13th edition).

[2] *Working principles for risk analysis for application in the framework of the Codex Alimentarius* (adopted by the 26th Session of the Codex Alimentarius Commission, 2003; *Codex Alimentarius Commission Procedural Manual*, 13th edition).

Adopted 2003. Amendment 2008.

SECTION 2 – SCOPE AND DEFINITIONS

7. The purpose of these Principles is to provide a framework for undertaking risk analysis on the safety and nutritional aspects of foods derived from modern biotechnology. This document does not address environmental, ethical, moral and socio-economic aspects of the research, development, production and marketing of these foods.[3]

8. The definitions below apply to these Principles:

Modern biotechnology means the application of:

i) *In vitro* nucleic acid techniques, including recombinant deoxyribonucleic acid (DNA) and direct injection of nucleic acid into cells or organelles; or

ii) fusion of cells beyond the taxonomic family that overcome natural physiological reproductive or recombinant barriers and that are not techniques used in traditional breeding and selection.[4]

Conventional counterpart means a related organism/variety, its components and/or products for which there is experience of establishing safety based on common use as food.[5]

SECTION 3 – PRINCIPLES

9. The risk analysis process for foods derived from modern biotechnology should be consistent with the *Working Principles for risk analysis*.

Risk assessment

10. Risk assessment includes a safety assessment, which is designed to identify whether a hazard, nutritional or other safety concern is present, and if present, to gather information on its nature and severity. The safety assessment should include a comparison between the food derived from modern biotechnology and its conventional counterpart, focusing on determination of similarities and differences. If a new or altered hazard, nutritional or other safety concern is identified by the safety assessment, the risk associated with it should be characterized to determine its relevance to human health.

11. A safety assessment is characterized by an assessment of a whole food or a component thereof relative to the appropriate conventional counterpart:

A. taking into account both intended and unintended effects;
B. identifying new or altered hazards;
C. identifying changes relevant to human health in key nutrients.

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[3] This document does not address animal feed and animals fed such feed except insofar as these animals have been developed by using modern biotechnology.

[4] This definition is taken from the *Cartagena Biosafety Protocol* under the Convention on Biological Diversity.

[5] It is recognized that, for the foreseeable future, foods derived from modern biotechnology will not be used as conventional counterparts.

12. A pre-market safety assessment should be undertaken following a structured and integrated approach and be performed on a case-by-case basis. The data and information, based on sound science, obtained using appropriate methods and analysed using appropriate statistical techniques, should be of a quality and, as appropriate, of a quantity that would withstand scientific peer review.

13. Risk assessment should apply to all relevant aspects of foods derived from modern biotechnology. The risk assessment approach for these foods is based on a consideration of science-based multidisciplinary data and information taking into account the factors mentioned in the accompanying Guidelines.[6]

14. Scientific data for risk assessment are generally obtained from a variety of sources, such as the developer of the product, scientific literature, general technical information, independent scientists, regulatory agencies, international bodies and other interested parties. Data should be assessed using appropriate science-based risk assessment methods.

15. Risk assessment should take into account all available scientific data and information derived from different testing procedures, provided that the procedures are scientifically sound and the parameters being measured are comparable.

Risk management

16. Risk management measures for foods derived from modern biotechnology should be proportional to the risk, based on the outcome of the risk assessment and, where relevant, taking into account other legitimate factors in accordance with the general decisions of the Codex Alimentarius Commission[7] as well as the *Working Principles for risk analysis*.

17. It should be recognized that different risk management measures may be capable of achieving the same level of protection with regard to the management of risks associated with safety and nutritional impacts on human health, and therefore would be equivalent.

18. Risk managers should take into account the uncertainties identified in the risk assessment and implement appropriate measures to manage these uncertainties.

19. Risk management measures may include, as appropriate, food labelling[8] conditions for marketing approvals and post-market monitoring.

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[6] Reference is made to the *Guideline for the conduct of food safety assessment of foods derived from recombinant-DNA plants* (CAC/GL 45-2003), the *Guideline for the conduct of food safety assessment of foods produced using recombinant-DNA micro-organisms* (CAC/GL 46-2003) and the *Guideline for the conduct of food safety assessment of foods derived from recombinant-DNA animals* (CAC/GL 68-2008).

[7] See footnote 1.

[8] Reference is made to the Codex Committee on Food Labelling (CCFL) in relation to the proposed *Draft Guidelines for the labelling of foods and food ingredients obtained through certain techniques of genetic modification/genetic engineering* at Step 3 of the Codex Elaboration Procedure.

20. Post-market monitoring may be an appropriate risk management measure in specific circumstances. Its need and utility should be considered, on a case-by-case basis, during risk assessment and its practicability should be considered during risk management. Post-market monitoring may be undertaken for the purpose of:

A. verifying conclusions about the absence or the possible occurrence, impact and significance of potential consumer health effects; and

B. monitoring changes in nutrient intake levels, associated with the introduction of foods likely to alter nutritional status significantly, to determine their human health impact.

21. Specific tools may be needed to facilitate the implementation and enforcement of risk management measures. These may include appropriate analytical methods; reference materials; and, the tracing of products[9] for the purpose of facilitating withdrawal from the market when a risk to human health has been identified or to support post-market monitoring in circumstances as indicated in paragraph 20.

Risk communication

22. Effective risk communication is essential in all phases of risk assessment and risk management. It is an interactive process involving all interested parties, including government, industry, academia, media and consumers.

23. Risk communication should include transparent safety assessment and risk management decision-making processes. These processes should be fully documented at all stages and open to public scrutiny, whilst respecting legitimate concerns to safeguard the confidentiality of commercial and industrial information. In particular, reports prepared on the safety assessments and other aspects of the decision-making process should be made available to all interested parties.

24. Effective risk communication should include responsive consultation processes. Consultation processes should be interactive. The views of all interested parties should be sought and relevant food safety and nutritional issues that are raised during consultation should be addressed during the risk analysis process.

Consistency

25. A consistent approach should be adopted to characterize and manage safety and nutritional risks associated with foods derived from modern biotechnology. Unjustified differences in the level of risks presented to consumers between these foods and similar conventional foods should be avoided.

[9] It is recognized that there are other applications of product tracing. These applications should be consistent with the provisions of the Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement) and the Agreement on Technical Barriers to Trade (TBT Agreement). The application of product tracing to the areas covered by both Agreements was considered by the Codex Committee on Food Import and Export Inspection and Certification Systems, see: *Principles for traceability / product tracing as a tool within a food inspection and certification system* (CAC/GL 60-2006).

26. A transparent and well-defined regulatory framework should be provided in characterizing and managing the risks associated with foods derived from modern biotechnology. This should include consistency of data requirements, assessment frameworks, the acceptable level of risk, communication and consultation mechanisms and timely decision processes.

Capacity building and information exchange

27. Efforts should be made to improve the capability of regulatory authorities, particularly those of developing countries, to assess, manage and communicate risks, including enforcement, associated with foods derived from modern biotechnology or to interpret assessments undertaken by other authorities or recognized expert bodies, including access to analytical technology. In addition, capacity building for developing countries, either through bilateral arrangements or with assistance of international organizations, should be directed towards effective application of these principles.[10]

28. Regulatory authorities, international organizations and expert bodies and industry should facilitate, through appropriate contact points (including but not limited to Codex Contact Points) and other appropriate means, the exchange of information, including the information on analytical methods.

Review processes

29. Risk analysis methodology and its application should be consistent with new scientific knowledge and other information relevant to risk analysis.

30. Recognizing the rapid pace of development in the field of biotechnology, the approach to safety assessments of foods derived from modern biotechnology should be reviewed when necessary to ensure that emerging scientific information is incorporated into the risk analysis. When new scientific information relevant to a risk assessment becomes available, the assessment should be reviewed to incorporate that information and, if necessary, risk management measures adapted accordingly.

[10] Reference is made to technical assistance of provisions in Article 9 of the SPS Agreement and Article 11 of the TBT Agreement.

GUIDELINE FOR THE CONDUCT OF FOOD SAFETY ASSESSMENT OF FOODS DERIVED FROM RECOMBINANT-DNA PLANTS

CAC/GL 45-2003

SECTION 1 – SCOPE

1. This Guideline supports the *Principles for the risk analysis of foods derived from modern biotechnology* (CAC/GL 44-2003). It addresses safety and nutritional aspects of foods consisting of, or derived from, plants that have a history of safe use as sources of food, and that have been modified by modern biotechnology to exhibit new or altered expression of traits.

2. This document does not address animal feed or animals fed with the feed. This document also does not address environmental risks.

3. The Codex principles of risk analysis, particularly those for risk assessment, are primarily intended to apply to discrete chemical entities, such as food additives and pesticide residues, or a specific chemical or microbial contaminant that have identifiable hazards and risks; they are not intended to apply to whole foods as such. Indeed, few foods have been assessed scientifically in a manner that would fully characterize all risks associated with the food. Further, many foods contain substances that would probably be found harmful if subjected to conventional approaches to safety testing. Thus, a more focused approach is required where the safety of a whole food is being considered.

4. This approach is based on the principle that the safety of foods derived from new plant varieties, including recombinant deoxyribonucleic acid (DNA) plants, is assessed relative to the conventional counterpart having a history of safe use, taking into account both intended and unintended effects. Rather than trying to identify every hazard associated with a particular food, the intention is to identify new or altered hazards relative to the conventional counterpart.

5. This safety assessment approach falls within the risk assessment framework as discussed in Section 3 of the *Principles for the risk analysis of foods derived from modern biotechnology* (CAC/GL 44-2003). If a new or altered hazard, nutritional or other food safety concern is identified by the safety assessment, the risk associated with it would first be assessed to determine its relevance to human health. Following the safety assessment and if necessary further risk assessment, the food would be subjected to risk management considerations in accordance with the *Principles for the risk analysis of foods derived from modern biotechnology* (CAC/GL 44-2003) before it is considered for commercial distribution.

Adopted 2003. Annexes 2 and 3 adopted 2008.

6. Risk management measures such as post-market monitoring of consumer health effects may assist the risk assessment process. These are discussed in paragraph 20 of the *Principles for the risk analysis of foods derived from modern biotechnology* (CAC/GL 44-2003).

7. The Guideline describes the recommended approach to making safety assessments of foods derived from recombinant-DNA plants where a conventional counterpart exists, and identifies the data and information that are generally applicable to making such assessments. While this Guideline is designed for foods derived from recombinant-DNA plants, the approach described could, in general, be applied to foods derived from plants that have been altered by other techniques.

SECTION 2 – DEFINITIONS

8. The definitions below apply to this Guideline:

Recombinant-DNA plant means a plant in which the genetic material has been changed through *in vitro* nucleic acid techniques, including recombinant deoxyribonucleic acid (DNA) and direct injection of nucleic acid into cells or organelles.

Conventional counterpart means a related plant variety, its components and/or products for which there is experience of establishing safety based on common use as food.[1]

SECTION 3 – INTRODUCTION TO FOOD SAFETY ASSESSMENT

9. Traditionally, new varieties of food plants have not been systematically subjected to extensive chemical, toxicological or nutritional evaluation prior to marketing, with the exception of foods for specific groups, such as infants, where the food may constitute a substantial portion of the diet. Thus, new varieties of corn, soybean, potatoes and other common food plants are evaluated by breeders for agronomic and phenotypic characteristics, but generally, foods derived from such new plant varieties are not subjected to the rigorous and extensive food safety testing procedures, including studies in animals, that are typical of chemicals, such as food additives or pesticide residues, that may be present in food.

10. The use of animal models for assessing toxicological end-points is a major element in the risk assessment of many compounds such as pesticides. However, in most cases, the substance to be tested is well characterized, of known purity, of no particular nutritional value, and human exposure to it is generally low. Therefore, it is relatively straightforward to feed such compounds to animals at a range of doses some several orders of magnitude greater than the expected human exposure levels in order to identify any potential adverse health effects of importance to humans. In this way, it is

[1] It is recognized that, for the foreseeable future, foods derived from modern biotechnology will not be used as conventional counterparts.

possible, in most cases, to estimate levels of exposure at which adverse effects are not observed and to set safe intake levels by the application of appropriate safety factors.

11. Animal studies cannot readily be applied to testing the risks associated with whole foods, which are complex mixtures of compounds, often characterized by a wide variation in composition and nutritional value. Owing to their bulk and effect on satiety, they can usually only be fed to animals at low multiples of the amounts that might be present in the human diet. In addition, a key factor to consider in conducting animal studies on foods is the nutritional value and balance of the diets used, this in order to avoid the induction of adverse effects that are not related directly to the material itself. Detecting any potential adverse effects and relating these conclusively to an individual characteristic of the food can, therefore, be extremely difficult. If the characterization of the food indicates that the available data are insufficient for a thorough safety assessment, properly designed animal studies could be requested on the whole foods. Another consideration in deciding the need for animal studies is whether it is appropriate to subject experimental animals to such a study if it is unlikely to give rise to meaningful information.

12. Owing to the difficulties of applying traditional toxicological testing and risk assessment procedures to whole foods, a more focused approach is required for the safety assessment of foods derived from food plants, including recombinant-DNA plants. This has been addressed by the development of a multidisciplinary approach for assessing safety that takes into account both intended and unintended changes that may occur in the plant or in the foods derived from it, using the concept of substantial equivalence.

13. The concept of substantial equivalence is a key step in the safety assessment process. However, it is not a safety assessment in itself; rather, it represents the starting point that is used to structure the safety assessment of a new food relative to its conventional counterpart. This concept is used to identify similarities and differences between the new food and its conventional counterpart.[2] It aids in the identification of potential safety and nutritional issues and is considered the most appropriate strategy to date for safety assessment of foods derived from recombinant-DNA plants. The safety assessment carried out in this way does not imply absolute safety of the new product; rather, it focuses on assessing the safety of any identified differences so that the safety of the new product can be considered relative to its conventional counterpart.

Unintended effects

14. In achieving the objective of conferring a specific target trait (intended effect) to a plant by the insertion of defined DNA sequences, additional traits could, in some cases, be acquired or existing traits could be lost or modified (unintended effects). The potential occurrence of unintended effects is not restricted to the use of *in vitro* nucleic acid techniques. Rather, it is an inherent and general phenomenon that can

[2] The concept of substantial equivalence as described in the report of the 2000 Joint FAO/WHO Expert Consultation (*Safety aspects of genetically modified foods of plant origin,* WHO/SDE/PHE/FOS/00.6, WHO, Geneva, 2000).

also occur in conventional breeding. Unintended effects may be deleterious, beneficial or neutral with respect to the health of the plant or the safety of foods derived from the plant. Unintended effects in recombinant-DNA plants may also arise through the insertion of DNA sequences and/or they may arise through subsequent conventional breeding of the recombinant-DNA plant. Safety assessment should include data and information to reduce the possibility that a food derived from a recombinant-DNA plant would have an unexpected adverse effect on human health.

15. Unintended effects can result from the random insertion of DNA sequences into the plant genome, which may cause disruption or silencing of existing genes, activation of silent genes, or modifications in the expression of existing genes. Unintended effects may also result in the formation of new or changed patterns of metabolites. For example, the expression of enzymes at high levels may give rise to secondary biochemical effects or changes in the regulation of metabolic pathways and/or altered levels of metabolites.

16. Unintended effects caused by genetic modification may be subdivided into two groups: those that are "predictable" and those that are "unexpected". Many unintended effects are largely predictable based on knowledge of the inserted trait and its metabolic connections or of the site of insertion. Owing to the expanding information on plant genomes and the increased specificity in terms of genetic materials introduced through recombinant-DNA techniques compared with other forms of plant breeding, it may become easier to predict unintended effects of a particular modification. Molecular biological and biochemical techniques can also be used to analyse potential changes at the level of gene transcription and message translation that could lead to unintended effects.

17. The safety assessment of foods derived from recombinant-DNA plants involves methods to identify and detect such unintended effects and procedures to evaluate their biological relevance and potential impact on food safety. A variety of data and information is necessary in order to assess unintended effects because no individual test can detect all possible unintended effects or identify, with certainty, those relevant to human health. These data and information, when considered in total, provide assurance that the food is unlikely to have an adverse effect on human health. The assessment for unintended effects takes into account the agronomic/phenotypic characteristics of the plant that are typically observed by breeders in selecting new varieties for commercialization. These observations by breeders provide a first screen for plants that exhibit unintended traits. New varieties that pass this screen are subjected to safety assessment as described in Sections 4 and 5.

Framework of food safety assessment

18. The safety assessment of a food derived from a recombinant-DNA plant follows a stepwise process of addressing relevant factors that include:

A. description of the recombinant-DNA plant;
B. description of the host plant and its use as food;
C. description of the donor organism(s);

D. description of the genetic modification(s);
E. characterization of the genetic modification(s);
F. safety assessment:
   a) expressed substances (non-nucleic acid substances),
   b) compositional analyses of key components,
   c) evaluation of metabolites,
   d) food processing,
   e) nutritional modification; and
G. other considerations.

19. In certain cases, the characteristics of the product may necessitate development of additional data and information to address issues that are unique to the product under review.

20. Experiments intended to develop data for safety assessments should be designed and conducted in accordance with sound scientific concepts and principles, as well as, where appropriate, good laboratory practice. Primary data should be made available to regulatory authorities at request. Data should be obtained using sound scientific methods and analysed using appropriate statistical techniques. The sensitivity of all analytical methods should be documented.

21. The goal of each safety assessment is to provide assurance, in the light of the best available scientific knowledge, that the food does not cause harm when prepared, used and/or eaten according to its intended use. The expected end-point of such an assessment will be a conclusion regarding whether the new food is as safe as the conventional counterpart taking into account dietary impact of any changes in nutritional content or value. In essence, therefore, the outcome of the safety assessment process is to define the product under consideration in such a way as to enable risk managers to determine whether any measures are needed and, if so, to make well-informed and appropriate decisions.

SECTION 4 – GENERAL CONSIDERATIONS

Description of the recombinant-DNA plant

22. A description of the recombinant-DNA plant being presented for safety assessment should be provided. This description should identify the crop, the transformation event(s) to be reviewed and the type and purpose of the modification. This description should be sufficient to aid in understanding the nature of the food being submitted for safety assessment.

Description of the host plant and its use as food

23. A comprehensive description of the host plant should be provided. The necessary data and information should include, but need not be restricted to:
A. common or usual name, scientific name and taxonomic classification;
B. history of cultivation and development through breeding, in particular identifying traits that may adversely affect human health;

C. information on the genotype and phenotype of the host plant relevant to its safety, including any known toxicity or allergenicity; and
D. history of safe use for consumption as food.

24. Relevant phenotypic information should be provided not only for the host plant but also for related species and for plants that have made or may make a significant contribution to the genetic background of the host plant.

25. The history of use may include information on how the plant is typically cultivated, transported and stored, whether special processing is required to make the plant safe to eat, and its normal role in the diet (e.g. which part of the plant is used as a food source, whether its consumption is important in particular subgroups of the population, what important macronutrients or micronutrients it contributes to the diet).

Description of the donor organism(s)

26. Information should be provided on the donor organism(s) and, when appropriate, on other related species. It is particularly important to determine if the donor organism(s) or other closely related members of the family naturally exhibit characteristics of pathogenicity or toxin production, or have other traits that affect human health (e.g. presence of antinutrients). The description of the donor organism(s) should include:
A. its usual or common name;
B. scientific name;
C. taxonomic classification;
D. information about the natural history as concerns food safety;
E. information on naturally occurring toxins, antinutrients and allergens; for micro-organisms, additional information on pathogenicity and the relationship to known pathogens; and
F. information on the past and present use, if any, in the food supply and exposure route(s) other than intended food use (e.g. possible presence as contaminants).

Description of the genetic modification(s)

27. Sufficient information should be provided on the genetic modification to allow for the identification of all genetic material potentially delivered to the host plant and to provide the necessary information for the analysis of the data supporting the characterization of the DNA inserted in the plant.

28. The description of the transformation process should include:
A. information on the specific method used for the transformation (e.g. *Agrobacterium*-mediated transformation);
B. information, if applicable, on the DNA used to modify the plant (e.g. helper plasmids), including the source (e.g. plant, microbial, viral, synthetic), identity and expected function in the plant; and
C. intermediate host organisms, including the organisms (e.g. bacteria) used to produce or process DNA for transformation of the host organism.

29. Information should be provided on the DNA to be introduced, including:
   A. the characterization of all the genetic components, including marker genes, regulatory and other elements affecting the function of the DNA;
   B. the size and identity;
   C. the location and orientation of the sequence in the final vector/construct; and
   D. the function.

Characterization of the genetic modification(s)

30. In order to provide clear understanding of the impact on the composition and safety of foods derived from recombinant-DNA plants, a comprehensive molecular and biochemical characterization of the genetic modification should be carried out.

31. Information should be provided on the DNA insertions into the plant genome; this should include:
   A. the characterization and description of the inserted genetic materials;
   B. the number of insertion sites;
   C. the organization of the inserted genetic material at each insertion site, including copy number and sequence data of the inserted material and of the surrounding region, sufficient to identify any substances expressed as a consequence of the inserted material, or, where more appropriate, other information such as analysis of transcripts or expression products to identify any new substances that may be present in the food; and
   D. identification of any open reading frames within the inserted DNA or created by the insertions with contiguous plant genomic DNA, including those that could result in fusion proteins.

32. Information should be provided on any expressed substances in the recombinant-DNA plant; this should include:
   A. the gene product(s) (e.g. a protein or an untranslated ribonucleic acid [RNA]);
   B. the function of the gene product(s);
   C. the phenotypic description of the new trait(s);
   D. the level and site of expression in the plant of the expressed gene product(s), and the levels of its metabolites in the plant, particularly in the edible portions; and
   E. where possible, the amount of the target gene product(s) if the function of the expressed sequence(s)/gene(s) is to alter the accumulation of a specific endogenous messenger RNA (mRNA) or protein.

33. In addition, information should be provided:
   A. to demonstrate whether the arrangement of the genetic material used for insertion has been conserved or whether significant rearrangements have occurred upon integration;
   B. to demonstrate whether deliberate modifications made to the amino acid sequence of the expressed protein result in changes in its post-translational modification or affect sites critical for its structure or function;

C. to demonstrate whether the intended effect of the modification has been achieved and that all expressed traits are expressed and inherited in a manner that is stable through several generations consistent with laws of inheritance. It may be necessary to examine the inheritance of the DNA insert itself or the expression of the corresponding RNA if the phenotypic characteristics cannot be measured directly;
D. to demonstrate whether the newly expressed trait(s) are expressed as expected in the appropriate tissues in a manner and at levels that are consistent with the associated regulatory sequences driving the expression of the corresponding gene;
E. to indicate whether there is any evidence to suggest that one gene (or several genes) in the host plant has been affected by the transformation process; and
F. to confirm the identity and expression pattern of any new fusion proteins.

Safety assessment

Expressed substances (non-nucleic acid substances)

Assessment of possible toxicity

34. *In vitro* nucleic acid techniques enable the introduction of DNA that can result in the synthesis of new substances in plants. The new substances can be conventional components of plant foods, such as proteins, fats, carbohydrates and vitamins, that are novel in the context of that recombinant-DNA plant. New substances might also include new metabolites resulting from the activity of enzymes generated by the expression of the introduced DNA.

35. The safety assessment should take into account the chemical nature and function of the newly expressed substance and identify the concentration of the substance in the edible parts of the recombinant-DNA plant, including variations and mean values. Current dietary exposure and possible effects on population subgroups should also be considered.

36. Information should be provided to ensure that genes coding for known toxins or antinutrients present in the donor organisms are not transferred to recombinant-DNA plants that do not normally express those toxic or antinutritious characteristics. This assurance is particularly important in cases where a recombinant-DNA plant is processed differently from a donor plant, as conventional food processing techniques associated with the donor organisms may deactivate, degrade or eliminate antinutrients or toxicants.

37. For the reasons described in Section 3, conventional toxicology studies may not be considered necessary where the substance or a closely related substance has, taking into account its function and exposure, been consumed safely in food. In other cases, the use of appropriate conventional toxicology or other studies on the new substance may be necessary.

38. In the case of proteins, the assessment of potential toxicity should focus on amino acid sequence similarity between the protein and known protein toxins and antinutrients (e.g. protease inhibitors, lectins) as well as stability to heat or processing and to degradation in appropriate representative gastric and intestinal model systems.

Appropriate oral toxicity studies[3] may need to be carried out in cases where the protein present in the food is not similar to proteins that have previously been consumed safely in food, and taking into account its biological function in the plant where known.

39. Potential toxicity of non-protein substances that have not been safely consumed in food should be assessed on a case-by-case basis depending on the identity and biological function in the plant of the substance and dietary exposure. The types of studies to be performed may include studies on metabolism, toxicokinetics, subchronic toxicity, chronic toxicity/carcinogenicity, reproduction and development toxicity according to the traditional toxicological approach.

40. This may require the isolation of the new substance from the recombinant-DNA plant, or the synthesis or production of the substance from an alternative source, in which case, the material should be shown to be biochemically, structurally and functionally equivalent to that produced in the recombinant-DNA plant.

Assessment of possible allergenicity (proteins)

41. When the protein(s) resulting from the inserted gene is present in the food, it should be assessed for potential allergenicity in all cases. An integrated, stepwise, case-by-case approach used in the assessment of the potential allergenicity of the newly expressed protein(s) should rely upon various criteria used in combination (as no single criterion is sufficiently predictive on either allergenicity or non-allergenicity). As noted in paragraph 20, the data should be obtained using sound scientific methods. A detailed presentation of issues to be considered can be found in Annex 1 to this document.[4]

42. The newly expressed proteins in foods derived from recombinant-DNA plants should be evaluated for any possible role in the elicitation of gluten-sensitive enteropathy if the introduced genetic material is obtained from wheat, rye, barley, oats or related cereal grains.

43. The transfer of genes from commonly allergenic foods and from foods known to elicit gluten-sensitive enteropathy in sensitive individuals should be avoided unless it is documented that the transferred gene does not code for an allergen or for a protein involved in gluten-sensitive enteropathy.

Compositional analyses of key components

44. Analyses of concentrations of key components[5] of the recombinant-DNA plant and, especially those typical of the food, should be compared with an equivalent analysis

[3] Guidelines for oral toxicity studies have been developed in international fora, for example, the *OECD Guidelines for the Testing of Chemicals* issued by the Organisation for Economic Co-operation and Development.

[4] The FAO/WHO Expert Consultation 2001 report, which includes reference to several decision trees, was used in developing Annex 1 to these Guidelines.

[5] Key nutrients or key antinutrients are those components in a particular food that may have a substantial impact in the overall diet. They may be major constituents (fats, proteins, carbohydrates as nutrients or enzyme inhibitors as antinutrients) or minor compounds (minerals, vitamins). Key toxicants are those toxicologically significant compounds known to be inherently present in the plant, such as those compounds whose toxic potency and level may be significant to health (e.g. solanine in potatoes if the level is increased, selenium in wheat) and allergens.

of a conventional counterpart grown and harvested under the same conditions. In some cases, a further comparison with the recombinant-DNA plant grown under its expected agronomic conditions may need to be considered (e.g. application of a herbicide). The statistical significance of any observed differences should be assessed in the context of the range of natural variations for that parameter to determine its biological significance. The comparator(s) used in this assessment should ideally be the near isogenic parental line. In practice, this may not be feasible at all times, in which case a line as close as possible should be chosen. The purpose of this comparison, in conjunction with an exposure assessment as necessary, is to establish that substances that are nutritionally important or that can affect the safety of the food have not been altered in a manner that would have an adverse impact on human health.

45. The location of trial sites should be representative of the range of environmental conditions under which the plant varieties would be expected to be grown. The number of trial sites should be sufficient to allow accurate assessment of compositional characteristics over this range. Similarly, trials should be conducted over a sufficient number of generations to allow adequate exposure to the variety of conditions met in nature. To minimize environmental effects, and to reduce any effect from naturally occurring genotypic variation within a crop variety, each trial site should be replicated. An adequate number of plants should be sampled and the methods of analysis should be sufficiently sensitive and specific to detect variations in key components.

Evaluation of metabolites

46. Some recombinant-DNA plants may have been modified in a manner that could result in new or altered levels of various metabolites in the food. Consideration should be given to the potential for the accumulation of metabolites in the food that would adversely affect human health. Safety assessment of such plants requires investigation of residue and metabolite levels in the food and assessment of any alterations in nutrient profile. Where altered residue or metabolite levels are identified in foods, consideration should be given to the potential impacts on human health using conventional procedures for establishing the safety of such metabolites (e.g. procedures for assessing the human safety of chemicals in foods).

Food processing

47. The potential effects of food processing, including home preparation, on foods derived from recombinant-DNA plants should also be considered. For example, alterations could occur in the heat stability of an endogenous toxicant or the bioavailability of an important nutrient after processing. Therefore, information should be provided, describing the processing conditions used in the production of a food ingredient from the plant. For example, in the case of vegetable oil, information should be provided on the extraction process and any subsequent refining steps.

Nutritional modification

48. The assessment of possible compositional changes to key nutrients, which should be conducted for all recombinant-DNA plants, has already been addressed under "Compositional analyses of key components". However, foods derived from

recombinant-DNA plants that have undergone modification to alter nutritional quality or functionality intentionally should be subjected to additional nutritional assessment in order 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. A detailed presentation of issues to be considered can be found in Annex 2 to this document.

49. Information about the known patterns of use and consumption of a food, and its derivatives should be used to estimate the likely intake of the food derived from the recombinant-DNA plant. The expected intake of the food should be used to assess the nutritional implications of the altered nutrient profile both at customary and maximal levels of consumption. Basing the estimate on the highest likely consumption provides assurance that the potential for any undesirable nutritional effects will be detected. Attention should be paid to the particular physiological characteristics and metabolic requirements of specific population groups, such as infants, children, pregnant and lactating women, the elderly and those with chronic diseases or compromised immune systems. Based on the analysis of nutritional impacts and the dietary needs of specific population subgroups, additional nutritional assessments may be necessary. It is also important to ascertain to what extent the modified nutrient is bioavailable and remains stable with time, processing and storage.

50. The use of plant breeding, including *in vitro* nucleic acid techniques, to change nutrient levels in crops can result in broad changes to the nutrient profile in two ways. The intended modification in plant constituents could change the overall nutrient profile of the plant product, and this change could affect the nutritional status of individuals consuming the food. Unexpected alterations in nutrients could have the same effect. Although the recombinant-DNA plant components may be individually assessed as safe, the impact of the change on the overall nutrient profile should be determined.

51. When the modification results in a food product, such as vegetable oil, with a composition that is significantly different from its conventional counterpart, it may be appropriate to use additional conventional foods or food components (i.e. foods or food components whose nutritional composition is closer to that of the food derived from recombinant-DNA plant) as appropriate comparators to assess the nutritional impact of the food.

52. Because of geographical and cultural variation in food consumption patterns, nutritional changes to a specific food may have a greater impact in some geographical areas or in some cultural populations than in others. Some food plants serve as the major source of a particular nutrient in some populations. The nutrient and the populations affected should be identified.

53. Some foods may require additional testing. For example, animal feeding studies may be warranted for foods derived from recombinant-DNA plants if changes in the bioavailability of nutrients are expected or if the composition is not comparable to conventional foods. In addition, foods designed for health benefits may require specific nutritional, toxicological or other appropriate studies. If the characterization

of the food indicates that the available data are insufficient for a thorough safety assessment, properly designed animal studies could be requested on the whole foods.

SECTION 5 – OTHER CONSIDERATIONS

Potential accumulation of substances significant to human health

54. Some recombinant-DNA plants may exhibit traits (e.g. herbicide tolerance) that may indirectly result in the potential for accumulation of pesticide residues, altered metabolites of such residues, toxic metabolites, contaminants, or other substances that may be relevant to human health. The safety assessment should take this potential for accumulation into account. Conventional procedures for establishing the safety of such compounds (e.g. procedures for assessing the human safety of chemicals) should be applied.

Use of antibiotic resistance marker genes

55. Alternative transformation technologies that do not result in antibiotic resistance marker genes in foods should be used in the future development of recombinant-DNA plants, where such technologies are available and demonstrated to be safe.

56. Gene transfer from plants and their food products to gut micro-organisms or human cells is considered a rare possibility because of the many complex and unlikely events that would need to occur consecutively. Nevertheless, the possibility of such events cannot be completely discounted.[6]

57. In assessing the safety of foods containing antibiotic resistance marker genes, the following factors should be considered:

A. the clinical and veterinary use and importance of the antibiotic in question;
(Certain antibiotics are the only drug available to treat some clinical conditions (e.g. vancomycin for use in treating certain staphylococcal infections). Marker genes encoding resistance to such antibiotics should not be used in recombinant-DNA plants.)

B. whether the presence in food of the enzyme or protein encoded by the antibiotic resistance marker gene would compromise the therapeutic efficacy of the orally administered antibiotic; and
(This assessment should provide an estimate of the amount of orally ingested antibiotic that could be degraded by the presence of the enzyme in food, taking into account factors such as dosage of the antibiotic, amount of enzyme likely to remain in food following exposure to digestive conditions, including neutral or alkaline stomach conditions and the need for enzyme cofactors, e.g. adenosine triphosphate [ATP] for enzymatic activity and estimated concentration of such factors in food.)

[6] In cases where there are high levels of naturally occurring bacteria that are resistant to the antibiotic, the likelihood of such bacteria transferring this resistance to other bacteria will be orders of magnitude higher than the likelihood of transfer between ingested foods and bacteria.

C. safety of the gene product, as would be the case for any other expressed gene product.

58. If evaluation of the data and information suggests that the presence of the antibiotic resistance marker gene or gene product presents risks to human health, the marker gene or gene product should not be present in the food. Antibiotic resistance genes used in food production that encode resistance to clinically used antibiotics should not be present in foods.

Review of safety assessments

59. The goal of the safety assessment is a conclusion as to whether the new food is as safe as the conventional counterpart, taking into account dietary impact of any changes in nutritional content or value. Nevertheless, the safety assessment should be reviewed in the light of new scientific information that calls into question the conclusions of the original safety assessment.

ANNEX 1

ASSESSMENT OF POSSIBLE ALLERGENICITY

SECTION 1 – INTRODUCTION

1. All newly expressed proteins[7] in recombinant-DNA plants that could be present in the final food should be assessed for their potential to cause allergic reactions. This should include consideration of whether a newly expressed protein is one to which certain individuals may already be sensitive as well as whether a protein new to the food supply is likely to induce allergic reactions in some individuals.

2. At present, there is no definitive test that can be relied upon to predict allergic response in humans to a newly expressed protein. Therefore, it is recommended that an integrated, stepwise, case-by-case approach, as described below, be used in the assessment of possible allergenicity of newly expressed proteins. This approach takes into account the evidence derived from several types of information and data as no single criterion is sufficiently predictive.

3. The end-point of the assessment is a conclusion as to the likelihood of the protein being a food allergen.

SECTION 2 – ASSESSMENT STRATEGY

4. The initial steps in assessing possible allergenicity of any newly expressed proteins are the determination of: the source of the introduced protein; any significant similarity between the amino acid sequence of the protein and that of known allergens; and its structural properties, including but not limited to, its susceptibility to enzymatic degradation, heat stability and/or acid and enzymatic treatment.

5. As there is no single test that can predict the likely human immunoglobulin E (IgE) response to oral exposure, the first step to characterize newly expressed proteins should be the comparison of the amino acid sequence and certain physicochemical characteristics of the newly expressed protein with those of established allergens in a weight of evidence approach. This will require the isolation of any newly expressed proteins from the recombinant-DNA plant, or the synthesis or production of the substance from an alternative source, in which case the material should be shown to be structurally, functionally and biochemically equivalent to that produced in the recombinant-DNA plant. Particular attention should be given to the choice of the expression host, as post-translational modifications allowed by different hosts (i.e.

[7] This assessment strategy is not applicable for assessing whether newly expressed proteins are capable of inducing gluten-sensitive or other enteropathies. The issue of enteropathies is already addressed in "Assessment of possible allergenicity (proteins)", paragraph 42 of the *Guideline for the conduct of food safety assessment of foods derived from recombinant-DNA plants*. In addition, the strategy is not applicable to the evaluation of foods where gene products are down regulated for hypoallergenic purposes.

eukaryotic vs prokaryotic systems) may have an impact on the allergenic potential of the protein.

6. It is important to establish whether the source is known to cause allergic reactions. Genes derived from known allergenic sources should be assumed to encode an allergen unless scientific evidence demonstrates otherwise.

SECTION 3 – INITIAL ASSESSMENT

Section 3.1 – Source of the protein

7. As part of the data supporting the safety of foods derived from recombinant-DNA plants, information should describe any reports of allergenicity associated with the donor organism. Allergenic sources of genes would be defined as those organisms for which reasonable evidence of IgE mediated oral, respiratory or contact allergy is available. Knowledge of the source of the introduced protein allows the identification of tools and relevant data to be considered in the allergenicity assessment. These include: the availability of sera for screening purposes; documented type, severity and frequency of allergic reactions; structural characteristics and amino acid sequence; physicochemical and immunological properties (when available) of known allergenic proteins from that source.

Section 3.2 – Amino acid sequence homology

8. The purpose of a sequence homology comparison is to assess the extent to which a newly expressed protein is similar in structure to a known allergen. This information may suggest whether that protein has an allergenic potential. Sequence homology searches comparing the structure of all newly expressed proteins with all known allergens should be done. Searches should be conducted using various algorithms such as FASTA or BLASTP to predict overall structural similarities. Strategies such as stepwise contiguous identical amino acid segment searches may also be performed for identifying sequences that may represent linear epitopes. The size of the contiguous amino acid search should be based on a scientifically justified rationale in order to minimize the potential for false negative or false positive results.[8] Validated search and evaluation procedures should be used in order to produce biologically meaningful results.

9. IgE cross-reactivity between the newly expressed protein and a known allergen should be considered a possibility when there is more than 35 percent identity in a segment of 80 or more amino acids (FAO/WHO, 2001) or other scientifically justified criteria. All the information resulting from the sequence homology comparison between the newly expressed protein and known allergens should be reported to allow a case-by-case scientifically-based evaluation.

[8] It is recognized that the 2001 FAO/WHO Consultation suggested moving from 8 to 6 identical amino acid segments in searches. The smaller the peptide sequence used in the stepwise comparison, the greater the likelihood of identifying false positives; inversely, the larger the peptide sequence used, the greater the likelihood of false negatives, thereby reducing the utility of the comparison.

10. Sequence homology searches have certain limitations. In particular, comparisons are limited to the sequences of known allergens in publicly available databases and the scientific literature. There are also limitations in the ability of such comparisons to detect non-contiguous epitopes capable of binding themselves specifically with IgE antibodies.

11. A negative sequence homology result indicates that a newly expressed protein is not a known allergen and is unlikely to be cross-reactive to known allergens. A result indicating absence of significant sequence homology should be considered along with the other data outlined under this strategy in assessing the allergenic potential of newly expressed proteins. Further studies should be conducted as appropriate (see also Sections 4 and 5). A positive sequence homology result indicates that the newly expressed protein is likely to be allergenic. If the product is to be considered further, it should be assessed using serum from individuals sensitized to the identified allergenic source.

Section 3.3 – Pepsin resistance

12. Resistance to pepsin digestion has been observed in several food allergens; thus, a correlation exists between resistance to digestion by pepsin and allergenic potential.[9] Therefore, the resistance of a protein to degradation in the presence of pepsin under appropriate conditions indicates that further analysis should be conducted to determine the likelihood of the newly expressed protein being allergenic. The establishment of a consistent and well-validated pepsin degradation protocol may enhance the utility of this method. However, it should be taken into account that a lack of resistance to pepsin does not exclude that the newly expressed protein can be a relevant allergen.

13. Although the pepsin resistance protocol is strongly recommended, it is recognized that other enzyme susceptibility protocols exist. Alternative protocols may be used where adequate justification is provided.[10]

SECTION 4 – SPECIFIC SERUM SCREENING

14. For those proteins that originate from a source known to be allergenic, or have sequence homology with a known allergen, testing in immunological assays should be performed where sera are available. Sera from individuals with a clinically validated allergy to the source of the protein can be used to test the specific binding to IgE class antibodies of the protein in *in vitro* assays. A critical issue for testing will be the availability of human sera from sufficient numbers of individuals.[11] In addition, the

[9] The method outlined in *The United States Pharmacopoeia* (1995) was used in the establishment of the correlation (Astwood *et al.*,1996).

[10] Report of the Joint FAO/WHO Expert Consultation on the allergenicity of foods derived from biotechnology (2001): *Evaluation of allergenicity of genetically modified foods,* Section 6.4 Pepsin resistance.

[11] According to the report of the Joint FAO/WHO Expert Consultation on allergenicity of foods derived from biotechnology (22–25 January 2001, Rome) a minimum of eight relevant sera is required in order to achieve a 99-percent certainty that the new protein is not an allergen in the case of a major allergen. Similarly, a minimum of 24 relevant sera is required to achieve the same level of certainty in the case of a minor allergen. It is recognized that these quantities of sera may not be available for testing purposes.

quality of the sera and the assay procedure need to be standardized to produce a valid test result. For proteins from sources not known to be allergenic and which do not exhibit sequence homology to a known allergen, targeted serum screening may be considered where such tests are available as described in paragraph 17.

15. In the case of a newly expressed protein derived from a known allergenic source, a negative result in *in vitro* immunoassays may not be considered sufficient, but should prompt additional testing, such as the possible use of skin test and *ex vivo* protocols.[12] A positive result in such tests would indicate a potential allergen.

SECTION 5 – OTHER CONSIDERATIONS

16. The absolute exposure to the newly expressed protein and the effects of relevant food processing will contribute towards an overall conclusion about the potential for human health risk. In this regard, the nature of the food product intended for consumption should be taken into consideration in determining the types of processing that would be applied and its effects on the presence of the protein in the final food product.

17. As scientific knowledge and technology evolve, other methods and tools may be considered in assessing the allergenicity potential of newly expressed proteins as part of the assessment strategy. These methods should be scientifically sound and may include: targeted serum screening (i.e. the assessment of binding to IgE in sera of individuals with clinically validated allergic responses to broadly related categories of foods); the development of international serum banks; use of animal models; and examination of newly expressed proteins for T-cell epitopes and structural motifs associated with allergens.

[12] *Ex vivo* procedure is described as the testing for allergenicity using cells or tissue culture from allergic human subjects (report of Joint FAO/WHO Expert Consultation on allergenicity of foods derived from biotechnology.

ANNEX 2

FOOD SAFETY ASSESSMENT OF FOODS DERIVED FROM RECOMBINANT-DNA PLANTS MODIFIED FOR NUTRITIONAL OR HEALTH BENEFITS

SECTION 1 – INTRODUCTION

1. General guidance for the safety assessment of foods derived from recombinant-DNA plants is provided in the *Guideline for the conduct of food safety assessment of foods derived from recombinant-DNA plants* (CAC/GL 45-2003) (Plant Guideline). This Annex provides additional considerations that are specific to foods modified for nutritional or health benefits. The document does not extend beyond a safety assessment and, therefore, it does not cover assessment of the benefits themselves or any corresponding health claims, or risk-management measures.[13]

2. The following factors determine whether a recombinant-DNA plant is a recombinant-DNA plant modified for nutritional or health benefits, and as such within the scope of this Annex:
   a) the recombinant-DNA plant exhibits a particular trait in portion(s) of the plant intended for food use; and
   b) the trait is a result of: (i) introduction of a new nutrient(s) or related substance(s), (ii) alteration of either the quantity or bioavailability of a nutrient(s) or related substance(s), (iii) removal or reduction of undesirable substance(s) (e.g. allergens or toxicants), or (iv) alteration of the interaction(s) of nutritional or health relevance of these substances.

SECTION 2 – DEFINITION

3. The definition below applies to this Annex:

   Nutrient[14] means any substance normally consumed as a constituent of food:
   a) that provides energy; or
   b) that is needed for growth and development and maintenance of healthy life; or
   c) a deficit of which will cause characteristic biochemical or physiological changes to occur.

4. This Annex draws, where appropriate, on the definitions of key nutritional concepts to be found or to be developed in relevant Codex texts, especially those elaborated by the Codex Committee on Nutrition and Foods for Special Dietary Uses.

[13] *Principles for the risk analysis of foods derived from modern biotechnology* (CAC/GL 44-2003, paragraph 19).
[14] *General Principles for the addition of essential nutrients to foods* (CAC/GL 09–1987).

SECTION 3 – FOOD SAFETY ASSESSMENT

5. The *General Principles for the addition of essential nutrients to foods* (CAC/GL 09-1987) are generally applicable to the assessment of food derived from a plant that is modified by increasing the amount of a nutrient(s) or related substance(s) available for absorption and metabolism. The food safety framework outlined within the Plant Guideline[15] applies to the overall safety assessment of a food derived from a recombinant-DNA plant modified for nutritional or health benefits. This Annex presents additional considerations regarding the food safety assessment of those foods.

6. Foods derived from recombinant-DNA plants modified for nutritional or health benefits may benefit certain populations/subpopulations, while other populations/subpopulations may be at risk from the same food.[16]

7. Rather than trying to identify every hazard associated with a particular food, the intention of a safety assessment of food derived from recombinant-DNA plants is the identification of new or altered hazards relative to the conventional counterpart.[17] As recombinant-DNA plants modified for nutritional or health benefits result in food products with a composition that may be significantly different from their conventional counterparts, the choice of an appropriate comparator[18] is of great importance for the safety assessment addressed in this Annex. Those alterations identified in a plant modified to obtain nutritional or health benefits are the subject of this safety assessment.

8. Upper levels of intake for many nutrients that have been set out by some national, regional and international bodies[19] may be considered, as appropriate. The basis for their derivation should also be considered in order to assess the public health implications of exceeding these levels.

9. The safety assessment of related substances should follow a case-by-case approach, taking into account upper levels as well as other values, where appropriate.

10. Although it is preferable to use a scientifically determined upper level of intake of a specific nutrient or related substance, when no such value has been determined, consideration may be given to an established history of safe use for nutrients or related substances that are consumed in the diet if the expected or foreseeable exposure would be consistent with those historical safe levels.

11. With conventional fortification of food, typically, a nutrient or a related substance is added at controlled concentrations and its chemical form is characterized. Levels of plant nutrients or related substances may vary in both conventionally bred and

[15] Paragraphs 18–21 and 48–53.
[16] Further guidance for susceptible and high-risk population groups is provided in paragraph 49 of the Plant Guideline.
[17] Plant Guideline, paragraph 4.
[18] Plant Guideline, paragraph 51.
[19] Where such guidance is not provided by Codex, information provided by FAO/WHO may be preferably considered.

recombinant-DNA plants owing to growing conditions. In addition, more than one chemical form of the nutrient might be expressed in the food as a result of the modification and these may not be characterized from a nutrition perspective. Where appropriate, information may be needed on the different chemical forms of the nutrient(s) or related substance(s) expressed in the portion of the plant intended for food use and their respective levels.

12. Bioavailability of the nutrient(s), related substance(s) or undesirable substance(s) in the food that were the subject of the modification in the recombinant-DNA plant should be established, where appropriate. If more than one chemical form of the nutrient(s) or related substance(s) is present, their combined bioavailability should be established, where appropriate.

13. Bioavailability will vary for different nutrients, and methods of testing for bioavailability should be relevant to the nutrient and the food containing the nutrient, as well as the health, nutritional status and dietary practices of the specific populations consuming the food. *In vitro* and *in vivo* methods to determine bioavailability exist, the latter conducted in animals and in humans. *In vitro* methods can provide information to assess extent of release of a substance from plant tissues during the digestive process. *In vivo* studies in animals are of limited value in assessing nutritional value or nutrient bioavailability for humans and would require careful design in order to be relevant. *In vivo* studies, in particular, human studies, may provide more relevant information about whether and to what extent the nutrient or related substance is bioavailable.

14. Guidance on dietary exposure assessment of foods derived from recombinant-DNA plants with nutritional modifications is provided in paragraph 49 of the Plant Guideline. In the context of this Annex, dietary exposure assessment is the estimation of the concentration of the nutrient(s) or related substance(s) in a food, the expected or foreseeable consumption of that food, and any known factors that influence bioavailability. Exposure to a nutrient(s) or related substance(s) should be evaluated in the context of the total diet and the assessment should be carried out based on the customary dietary consumption by the relevant population(s) of the corresponding food that is likely to be displaced. When evaluating the exposure, it is appropriate to consider information on whether the consumption of the modified food could lead to adverse nutritional effects as compared with consumption of the food that it is intended to replace. Most, if not all, aspects of exposure assessment are not unique to recombinant-DNA plants modified for nutritional or health benefits.[20]

15. The first step of an exposure assessment is determining the level(s) of the substance(s) in question in the portion of the plant intended for food use. Guidance on determining changes in levels of these substances is provided in the Plant Guideline.[21]

[20] Additional applicable guidance on dietary exposure assessment of nutrients and related substances is provided in the report of the Joint FAO/WHO Technical Workshop on nutrient risk assessment: *A model for establishing upper levels of intake for nutrients and related substances*, WHO Headquarters, Geneva, Switzerland, 2–6 May 2005.

[21] Paragraphs 44 and 45.

16. Consumption patterns will vary from country to country depending on the importance of the food in the diet(s) of a given population(s). Therefore, it is recommended that consumption estimates are based on national or regional food consumption data when available, using existing guidance on estimation of exposure in a given population(s).[22] When national or regional food consumption data are unavailable, food availability data may provide a useful resource.[23]

17. To assess the safety of a food derived from a recombinant-DNA plant modified for a nutritional or health benefit, the estimated intake of the nutrient or related substance in the population(s) is compared with the nutritional or toxicological reference values, such as upper levels of intake, acceptable daily intakes (ADIs) for that nutrient or related substance, where these values exist. This may involve assessments of different consumption scenarios against the relevant nutritional reference value, taking into account possible changes in bioavailability, or extend to probabilistic methods that characterize the distribution of exposures within the relevant population(s).

[22] *A model for establishing upper levels of intake for nutrients and related substances*. Report of the Joint FAO/WHO Technical Workshop on nutrient risk assessment. WHO Headquarters, Geneva, Switzerland, 2–6 May 2005.

[23] Data on staple food products may also be supplemented by information from FAO Food Balance Sheets.

ANNEX 3

FOOD SAFETY ASSESSMENT IN SITUATIONS OF LOW-LEVEL PRESENCE OF RECOMBINANT-DNA PLANT MATERIAL IN FOOD

SECTION 1 – PREAMBLE

1. An increasing number of recombinant-DNA plants are being authorized for commercialization. However, they are authorized at different rates in different countries. As a consequence of these asymmetric authorizations, low levels of recombinant-DNA plant materials that have passed a food safety assessment according to the *Guideline for the conduct of food safety assessment of foods derived from recombinant-DNA plants* (CAC/GL 45-2003) (Plant Guideline) in one or more countries may on occasion be present in food in importing countries in which the food safety of the relevant recombinant-DNA plants has not been determined.

2. This Annex describes the recommended approach to the food safety assessment in such situations of low-level presence of recombinant-DNA plant material or in advance preparation for such potential circumstances.[24]

3. This Annex also describes data- and information-sharing mechanisms to facilitate utilization of the Annex and to determine whether it should apply.

4. This Annex can be applied in two different dietary exposure situations:
   a) That involving commodities, such as grains, beans or oilseeds, in which exposure to food from a variety not authorized in the importing country would likely be to dilute low-level amounts at any one time. This would probably be the more common situation of low-level presence of recombinant-DNA plant material. Because any food-serving of grains, beans or oilseeds would almost necessarily come from multiple plants, and because of how these types of commodities generally are sourced from multiple farms, are commingled in grain elevators, are further commingled in export shipments, at import and when used in processed foods, any inadvertently commingled material derived from recombinant-DNA plant varieties would be present only at a low level in any individual serving of food.
   b) That involving foods that are commonly consumed whole and undiluted, such as some fruits and vegetables like potatoes, tomatoes, and papaya, in which exposure would be rare but could be to an undiluted form of the unauthorized recombinant-DNA plant material. While the likelihood of consuming material from such an unauthorized variety would be low and the likelihood of repeated consumption would be much lower, any such consumption might be of an entire unauthorized fruit or vegetable.

[24] This guidance is not intended for a recombinant-DNA plant that was not authorized in an importing country as a result of food safety assessment by that country.

5. In both cases, the dietary exposure will be significantly lower than would be considered in a food safety assessment of the recombinant-DNA plant according to the Plant Guideline. As a result, only certain elements of the Plant Guideline will be relevant and, therefore, are included in this Annex.

6. This Annex does not:
- address risk management measures; national authorities will determine when a recombinant-DNA plant material is present at a level low enough for this Annex to be appropriate;
- preclude national authorities from conducting a safety assessment according to the Plant Guideline; countries can decide when and how to use the Annex within the context of their regulatory systems; or
- eliminate the responsibility of industries, exporters and, when applicable, national competent authorities to continue to meet the relevant import requirements set by countries, including in relation to unauthorized recombinant-DNA plant material.

SECTION 2 – GENERAL AND OTHER CONSIDERATIONS

7. For the food safety assessment in situations of low-level presence of recombinant DNA plant materials in food, Sections 4 and 5 of the Plant Guideline apply as amended as follows. The applicable paragraphs are specifically indicated. Those paragraphs of the Plant Guidelines that are not listed can be omitted from consideration.

Description of the recombinant-DNA plant

8. Paragraph 22 of the Plant Guideline applies.

Description of the host plant and its use as a food

9. Paragraphs 23, 24 and 25 of the Plant Guideline apply.

Description of the donor organism(s)

10. Information should be provided on the donor organism(s) and, when appropriate, on other related species. It is particularly important to determine if the donor organism(s) or other closely related members of the family naturally exhibit characteristics of pathogenicity or toxin production, or have other traits that affect human health. The description of the donor organism(s) should include:

A. its usual or common name;
B. scientific name;
C. taxonomic classification;
D. information about the natural history as concerns food safety;
E. information on naturally occurring toxins and allergens; for micro-organisms, additional information on pathogenicity and the relationship to known pathogens; and

F. information on past and present use, if any, in the food supply and exposure route(s) other than intended food use (e.g. possible presence as contaminants).[25]

Description of the genetic modification(s)

11. Paragraphs 27, 28 and 29 of the Plant Guideline apply.

Characterization of the genetic modification(s)

12. Paragraphs 30 and 31 of the Plant Guideline apply.

13. Information should be provided on any expressed substances in the recombinant-DNA plant; this should include:
   A. the gene product(s) (e.g. a protein or an untranslated RNA);
   B. the function of the gene product(s);
   C. the phenotypic description of the new trait(s);
   D. the level and site of expression in the plant of the expressed gene product(s), and the levels of its metabolites in the edible portions of the plant; and
   E. where possible, the amount of the target gene product(s) if the function of the expressed sequence(s)/gene(s) is to alter the accumulation of a specific endogenous mRNA or protein.[26]

14. Paragraph 33 of the Plant Guideline applies.

Safety assessment

Expressed substances (non-nucleic acid substances)

Assessment of possible toxicity

15. The safety assessment should take into account the chemical nature and function of the newly expressed substance and identify the concentration of the substance in the edible parts of the recombinant-DNA plant, including variations and mean values.[27]

16. Information should be provided to ensure that genes coding for known toxins present in the donor organisms are not transferred to recombinant-DNA plants that do not normally express those toxic characteristics. This assurance is particularly important in cases where a recombinant-DNA plant is processed differently from a donor plant, as conventional food processing techniques associated with the donor organisms may deactivate, degrade or eliminate toxicants.[28]

17. Paragraph 37 of the Plant Guideline applies.

18. In the case of proteins, the assessment of potential toxicity should focus on amino acid sequence similarity between the protein and known protein toxins as well as stability to heat or processing and to degradation in appropriate representative gastric and

[25] The text of this paragraph was adapted from paragraph 26 of the Plant Guideline.
[26] The text of this paragraph was adapted from paragraph 32 of the Plant Guideline.
[27] The text of this paragraph was adapted from paragraph 35 of the Plant Guideline.
[28] The text of this paragraph was adapted from paragraph 36 of the Plant Guideline.

intestinal model systems. Appropriate oral toxicity studies[29] may need to be carried out in cases where the protein present in the food is not similar to proteins that have previously been consumed safely in food, and taking into account its biological function in the plant where known.[30]

19. Paragraphs 39 and 40 of the Plant Guideline apply.

Assessment of possible allergenicity (proteins)

20. Paragraphs 41, 42 and 43 of the Plant Guideline apply.

Analyses of key toxicants and allergens

21. Analyses of key toxicants[31] and allergens are important in certain cases of foods from recombinant-DNA plants (e.g. those that are commonly consumed whole and undiluted, such as potatoes, tomatoes, and papaya). Analyses of concentrations of key toxicants and allergens of the recombinant-DNA plant typical of the food should be compared with an equivalent analysis of a conventional counterpart grown and harvested under the same conditions. The statistical significance of any observed differences should be assessed in the context of the range of natural variations for that parameter to determine its biological significance. The comparator(s) used in this assessment should ideally be the near isogenic parental line. In practice, this may not be feasible at all times, in which case a line as close as possible should be chosen. The purpose of this comparison is to establish that substances that can affect the safety of the food have not been altered in a manner that would have an adverse impact on human health.[32]

22. The location of trial sites should be representative of the range of environmental conditions under which the plant varieties would be expected to be grown. The number of trial sites should be sufficient to allow accurate assessment of key toxicants and allergens over this range. Similarly, trials should be conducted over a sufficient number of generations to allow adequate exposure to the variety of conditions met in nature. To minimize environmental effects, and to reduce any effect from naturally occurring genotypic variation within a crop variety, each trial site should be replicated. An adequate number of plants should be sampled and the methods of analysis should be sufficiently sensitive and specific to detect variations in key toxicants and allergens.[33]

Evaluation of metabolites

23. Some recombinant-DNA plants may have been modified in a manner that could result in new or altered levels of various metabolites in the food. In certain cases of foods from recombinant-DNA plants (e.g. those that are commonly consumed whole

[29] Guidelines for oral toxicity studies have been developed in international fora, for example, the *OECD Guidelines for the Testing of Chemicals* issued by the Organisation for Economic Co-operation and Development.

[30] The text of this paragraph was adapted from paragraph 38 of the Plant Guideline.

[31] Key toxicants are those toxicologically significant compounds known to be inherently present in the plant, such as those compounds whose toxic potency and level may be significant to health (e.g. solanine in potatoes if the level is increased).

[32] The text of this paragraph was adapted from paragraph 44 of the Plant Guideline.

[33] The text of this paragraph was adapted from paragraph 45 of the Plant Guideline.

and undiluted), consideration should be given to the potential for the accumulation of metabolites in the food that would adversely affect human health. Food safety assessment in situations of low-level presence of recombinant-DNA material in foods from such plants requires investigation of residue and metabolite levels in the food. Where altered residue or metabolite levels are identified in foods, consideration should be given to the potential impacts on human health using conventional procedures for establishing the safety of such metabolites (e.g. procedures for assessing the human safety of chemicals in foods).[34]

Food processing

24. The potential effects of food processing, including home preparation, on foods derived from recombinant-DNA plants should also be considered. For example, alterations could occur in the heat stability of an endogenous toxicant. Therefore, information should be provided describing the processing conditions used in the production of a food ingredient from the plant. For example, in the case of vegetable oil, information should be provided on the extraction process and any subsequent refining steps.[35]

Potential accumulation of substances significant to human health

25. Some recombinant-DNA plants may exhibit traits (e.g. herbicide tolerance) that may indirectly result in the potential for accumulation of pesticide residues, altered metabolites of such residues, toxic metabolites, contaminants, or other substances that may be relevant to human health. In certain cases of foods from recombinant-DNA plants (e.g. those that are commonly consumed whole and undiluted), the risk assessment should take this potential for accumulation into account. Conventional procedures for establishing the safety of such compounds (e.g. procedures for assessing the human safety of chemicals) should be applied.[36]

Use of antibiotic resistance marker genes

26. Paragraphs 55, 56, 57 and 58 of the Plant Guideline apply.

SECTION 3 – GUIDANCE ON DATA AND INFORMATION SHARING

27. In order for Codex Members to use this Annex, it is essential that they have access to requisite data and information.

28. Codex Members should make available to a publicly accessible central database to be maintained by FAO information on recombinant-DNA plants authorized in accordance with the Plant Guideline. This information should be presented in accordance with the following format:
    a) name of product applicant;
    b) summary of application;
    c) country of authorization;

[34] The text of this paragraph was adapted from paragraph 46 of the Plant Guideline.
[35] The text of this paragraph was adapted from paragraph 47 of the Plant Guideline.
[36] The text of this paragraph was adapted from paragraph 54 of the Plant Guideline.

d) date of authorization;
e) scope of authorization;
f) unique identifier;
g) links to the information on the same product in other databases maintained by relevant international organizations, as appropriate;
h) summary of the safety assessment, which should be consistent with the framework of food safety assessment of the Plant Guideline;
i) where detection method protocols and appropriate reference material (non-viable or, in certain circumstances, viable) suitable for low-level situation may be obtained[37]; and
j) contact details of the competent authority(s) responsible for the safety assessment and the product applicant.

29. This process should facilitate rapid access by importing Codex Members to additional information relevant to the assessment of food safety assessment in situations of low-level presence of recombinant-DNA plant material in foods in accordance with this Annex.

30. The authorizing Codex Members should make available complementary information to other Codex Members on its safety assessment in accordance with the Plant Guideline, in conformity with its regulatory/legal framework.

31. The product applicant should provide further information and clarification as necessary to allow the assessment according to this Annex to proceed, as well as a validated protocol for an event-specific or trait-specific detection method suitable for low-level situations and appropriate reference materials (non-viable or, in certain circumstances, viable). This is without prejudice to legitimate concerns to safeguard the confidentiality of commercial and industrial information.

32. As appropriate, new scientific information relevant to the conclusions of the food safety assessment conducted in accordance with the Plant Guideline by the authorizing Codex Member should be made available.

[37] This information may be provided by the product applicant or in some cases by Codex Members.

GUIDELINE FOR THE CONDUCT OF FOOD SAFETY ASSESSMENT OF FOODS PRODUCED USING RECOMBINANT-DNA MICRO-ORGANISMS

CAC/GL 46-2003

SECTION 1 – SCOPE

1. This Guideline supports the *Principles for the risk analysis of foods derived from modern biotechnology* (CAC/GL 44-2003) and addresses safety and nutritional aspects of foods produced through the actions of recombinant deoxyribonucleic acid (recombinant-DNA) micro-organisms.[1] The recombinant-DNA micro-organisms that are used to produce these foods are typically derived using the techniques of modern biotechnology from strains that have a history of safe, purposeful use in food production. However, in instances where the recipient strains do not have a history of safe use, their safety will have to be established.[2] Such food and food ingredients may contain viable or non-viable recombinant-DNA micro-organisms or may be produced by fermentation using recombinant-DNA micro-organisms from which the recombinant-DNA micro-organisms may have been removed.

2. Recognizing that the following issues may have to be addressed by other bodies or other instruments, this document does not address:
   - safety of micro-organisms used in agriculture (for plant protection, biofertilizers, in animal feed or food derived from animals fed the feed, etc.);
   - risks related to environmental releases of recombinant-DNA micro-organisms used in food production;
   - safety of substances produced by micro-organisms that are used as additives or processing aids, including enzymes for use in food production;[3]
   - specific purported health benefits or probiotic effects that may be attributed to the use of micro-organisms in food; or
   - issues relating to the safety of food production workers handling recombinant-DNA micro-organisms.

3. A variety of micro-organisms used in food production have a long history of safe use that predates scientific assessment. Few micro-organisms have been assessed scientifically in a manner that would fully characterize all potential risks associated with the food they are used to produce, including, in some instances, the consumption of viable micro-

[1] The micro-organisms included in these applications are bacteria, yeasts and filamentous fungi. (Such uses could include, but are not limited to, production of yogurt, cheese, fermented sausages, natto, kimchi, bread, beer and wine.)

[2] The criterion for establishing the safety of micro-organisms used in the production of foods where there is no history of safe use is beyond the scope of the current document.

[3] The Joint FAO/WHO Expert Committee on Food Additives (JECFA) is revising guidelines for *General specifications and considerations for enzyme preparations used in food processing*. These guidelines have been used to evaluate enzyme preparations derived from genetically modified micro-organisms.

organisms. Furthermore, the Codex Principles of risk analysis, particularly those for risk assessment, are primarily intended to apply to discrete chemical entities such as food additives and pesticide residues, or specific chemical or microbial contaminants that have identifiable hazards and risks; they were not originally intended to apply to intentional uses of micro-organisms in food processing or in the foods transformed by microbial fermentations. The safety assessments that have been conducted have focused primarily on the absence of properties associated with pathogenicity in these micro-organisms and the absence of reports of adverse events attributed to ingestion of these micro-organisms, rather than evaluating the results of prescribed studies. Further, many foods contain substances that would be considered harmful if subjected to conventional approaches to safety testing. Thus, a more focused approach is required where the safety of a whole food is being considered.

4. Information considered in developing this approach includes:
   A. uses of living micro-organisms in food production;
   B. consideration of the types of genetic modifications likely to have been made in these organisms;
   C. the types of methodologies available for performing a safety assessment; and
   D. issues specific to the use of the recombinant-DNA micro-organism in food production, including its genetic stability, potential for gene transfer, colonization of the gastrointestinal tract and persistence[4] therein, interactions that the recombinant-DNA micro-organism may have with the gastrointestinal flora or the mammalian host, and any impact of the recombinant-DNA micro-organism on the immune system.

5. This approach is based on the principle that the safety of foods produced using recombinant-DNA micro-organisms is assessed relative to the conventional counterparts that have a history of safe use, not only for the food produced using a recombinant-DNA micro-organism, but also for the micro-organism itself. This approach takes both intended and unintended effects into account. Rather than trying to identify every hazard associated with a particular food or the micro-organism, the intention is to identify new or altered hazards relative to the conventional counterpart.

6. This safety assessment approach falls within the risk assessment framework as discussed in Section 3 of the *Principles for the risk analysis of foods derived from modern biotechnology* (CAC/GL 44-2003). If a new or altered hazard, nutritional or other food safety concern is identified by the safety assessment, the risk associated with it would first be assessed to determine its relevance to human health. Following the safety assessment and, if necessary, further risk assessment, the food or component of food, such as a micro-organism used in production, would be subjected to risk management considerations in accordance with the *Principles for the risk analysis of foods derived*

[4] Persistence connotes survival of micro-organisms in the gastrointestinal tract longer than two intestinal transit times (International Life Science Institute, *The safety assessment of viable genetically modified micro-organisms used as food,* 1999, Brussels; the Joint FAO/WHO Expert Consultation on Foods Derived from Biotechnology, *Safety assessment of foods derived from genetically modified micro-organisms*, 24–28 September 2001, Geneva, Switzerland).

from modern biotechnology (CAC/GL 44-2003) before it is considered for commercial distribution.

7. Risk management measures, such as post-market monitoring of consumer health effects, may assist the risk assessment process. These are discussed in paragraph 20 of the *Principles for the risk analysis of foods derived from modern biotechnology* (CAC/GL 44-2003).

8. The Guideline describes approaches recommended for making safety assessments of foods produced using recombinant-DNA micro-organisms, using comparison with a conventional counterpart. The safety assessment will focus on the safety of the recombinant-DNA micro-organisms used in food production and, where appropriate, on metabolites produced by the action of recombinant-DNA micro-organisms on food. The Guideline identifies the data and information that are generally applicable to making such assessments. When conducting a comparison of a recombinant-DNA micro-organism or a food produced using recombinant-DNA micro-organism with their respective conventional counterparts, any identified differences should be taken into account, whether they are the result of intended or unintended effects. Due consideration should be given to the interactions of the recombinant-DNA micro-organism with the food matrix or the microflora and to the safety of any newly expressed protein(s) and secondary metabolic products. While this Guideline is designed for foods produced using recombinant-DNA micro-organisms or their components, the approach described could, in general, be applied to foods produced using micro-organisms that have been altered by other techniques.

SECTION 2 – DEFINITIONS

9. The definitions below apply to this Guideline:

Recombinant-DNA micro-organism means bacteria, yeasts or filamentous fungi in which the genetic material has been changed through *in vitro* nucleic acid techniques including recombinant deoxyribonucleic acid (DNA) and direct injection of nucleic acid into cells or organelles.

Conventional counterpart[5] means:

- a micro-organism/strain with a known history of safe use in producing and/or processing the food and related to the recombinant-DNA strain. The micro-organism may be viable in the food or may be removed in processing or rendered non-viable during processing; or
- food produced using the traditional food production micro-organisms for which there is experience of establishing safety based on common use in food production.

[5] It is recognized that, for the foreseeable future, micro-organisms derived from modern biotechnology will not be used as conventional counterparts.

SECTION 3 – INTRODUCTION TO FOOD SAFETY ASSESSMENT

10. Most foods produced as a result of the purposeful growth of micro-organisms have their origins in antiquity, and have been deemed safe since long before the emergence of scientific methods for assessing safety. Micro-organisms possess properties, such as fast growth rates, that enable genetic modifications, whether employing conventional techniques or modern biotechnology, to be implemented in short time frames. Micro-organisms used in food production derived using conventional genetic techniques have not customarily been systematically subjected to extensive chemical, toxicological, epidemiological or medical evaluations prior to marketing. Instead, microbiologists, mycologists and food technologists have evaluated new strains of bacteria, yeasts and filamentous fungi for phenotypic characteristics that are useful in relation to food production.

11. Safety assessments of recombinant-DNA micro-organisms should document the use of related micro-organisms in foods, the absence of properties known to be characteristic of pathogens in the recombinant-DNA micro-organisms or the recipient strains used for constructing the recombinant-DNA micro-organisms, and known adverse events involving the recipient or related organisms. In addition, when a recombinant DNA micro-organism directly affects or remains in the food, any effects on the safety of the food should be examined.

12. The use of animal models for assessing toxicological effects is a major element in the risk assessment of many compounds, such as pesticides. However, in most cases, the substance to be tested is well characterized, of known purity, of no particular nutritional value, and human exposure to it is generally low. Therefore, it is relatively straightforward to feed such compounds to animals at a range of doses some several orders of magnitude greater than the expected human exposure levels, in order to identify any potential adverse health effects of importance to humans. In this way, it is possible, in most cases, to estimate levels of exposure at which adverse effects are not observed and to set safe intake levels by the application of appropriate safety factors.

13. Animal studies cannot readily be applied to testing the risks associated with whole foods, which are complex mixtures of compounds and often characterized by a wide variation in composition and nutritional value. Owing to their bulk and effect on satiety, they can usually only be fed to animals at low multiples of the amounts that might be present in the human diet. In addition, a key factor to consider in conducting animal studies on foods is the nutritional value and balance of the diets used, in order to avoid the induction of adverse effects that are not related directly to the material itself. Detecting any potential adverse effects and relating these conclusively to an individual characteristic of the food can, therefore, be extremely difficult. If the characterization of the food indicates that the available data are insufficient for a thorough safety assessment, properly designed animal studies could be requested on the whole food. Another consideration in deciding the need for animal studies is whether it is appropriate to subject experimental animals to such a study if it is unlikely to give rise to meaningful information.

14. Animal studies typically employed in toxicological evaluations also cannot be readily applied to testing potential risks associated with ingestion of micro-organisms used for food production. Micro-organisms are living entities, containing complex structures composed of many biochemicals and, therefore, are not comparable with pure compounds. In some processed foods, they can survive processing and ingestion and can compete and, in some cases, be retained in the intestinal environment for significant periods of time. Appropriate animal studies should be used to evaluate the safety of recombinant-DNA micro-organisms where the donor or the gene or gene product do not have a history of safe use in food, taking into account available information regarding the donor and the characterization of the modified genetic material and the gene product. Further, appropriately designed studies in animals may be used to assess the nutritional value of the food or the bioavailability of the newly expressed substance in the food.

15. Owing to the difficulties of applying traditional toxicological testing and risk assessment procedures to whole foods, a more focused approach is required for the safety assessment of foods produced using recombinant-DNA micro-organisms. This has been addressed by the development of a multidisciplinary approach for assessing safety that takes into account the intended effect, the nature of the modification and detectable unintended changes that may occur in the micro-organism or in its action on the food, using the concept of substantial equivalence.[6]

16. While the focus of a safety assessment will be on the recombinant-DNA micro-organism, additional information on its interaction with the food matrix should be taken into consideration when applying the concept of substantial equivalence, which is a key step in the safety assessment process. However, the concept of substantial equivalence is not a safety assessment in itself. Rather it represents the starting point that is used to structure the safety assessment of both a recombinant-DNA micro-organism relative to its conventional counterpart and the food produced using a recombinant-DNA micro-organism relative to its conventional counterpart. This concept is used to identify for evaluation similarities and differences between recombinant-DNA micro-organisms used in food processing as well as the food produced using the recombinant-DNA micro-organisms and their respective conventional counterparts as defined in paragraph 9. It aids in the identification of potential safety and nutritional issues and is considered the most appropriate strategy to date for safety assessment of foods produced using recombinant-DNA micro-organisms. The safety assessment carried out in this way does not imply absolute safety of the new product; rather, it focuses on assessing the safety of any identified differences so that the safety of the recombinant-DNA micro-organism and the food produced using the recombinant-DNA micro-organism can be considered relative to their respective conventional counterparts.

[6] The concept of *substantial equivalence* as described in the Joint FAO/WHO Expert Consultation on foods derived from biotechnology, *Safety aspects of genetically modified plants,* 29 May – 2 June 2000, Geneva, Switzerland, and Section 4.3 of the Joint FAO/WHO Expert Consultation of Foods Derived from Biotechnology, *Safety assessment of foods derived from genetically modified micro-organisms*, 24–28 September 2001, Geneva, Switzerland.

Unintended effects

17. In achieving the objective of conferring a specific target trait (intended effect) to a micro-organism by the addition, substitution, removal or rearrangement of defined DNA sequences, including those used for the purpose of DNA transfer or maintenance in the recipient organism, additional traits could, in some cases, be acquired or existing traits could be lost or modified. The potential for occurrence of unintended effects is not restricted to the use of *in vitro* nucleic acid techniques. Rather, it is an inherent and general phenomenon that can also occur in the development of strains using traditional genetic techniques and procedures, or from exposure of micro-organisms to intentional or unintended selective pressures. Unintended effects may be deleterious, beneficial or neutral with respect to competition with other micro-organisms, ecological fitness of the micro-organism, the effects of the micro-organism on humans after ingestion, or the safety of foods produced using the micro-organism. Unintended effects in recombinant-DNA micro-organisms may also arise through intentional modification of DNA sequences or they may arise through recombination or other natural events in the recombinant-DNA micro-organism. Safety assessment should include data and information to reduce the possibility that a food derived from a recombinant-DNA micro-organism would have an unexpected, adverse effect on human health.

18. Unintended effects can result from the insertion of DNA sequences new to a micro-organism into the microbial genome; they may be compared with those observed following the activity of naturally occurring transposable genetic elements. Insertion of DNA may lead to changes in expression of genes in the genome of the recipient. The insertion of DNA from heterologous sources into a gene may also result in the synthesis of a chimeric protein, also referred to as a fusion protein. In addition, genetic instability and its consequences need to be considered.

19. Unintended effects may also result in the formation of new or changed patterns of metabolites. For example, the expression of enzymes at high levels or the expression of an enzyme new to the organism may give rise to secondary biochemical effects, changes in the regulation of metabolic pathways or altered levels of metabolites.

20. Unintended effects caused by to genetic modification may be subdivided into two groups: those that could be predicted, and those that are "unexpected". Many unintended effects are largely predictable based on knowledge of the added trait, its metabolic consequences or the site of insertion. As a consequence of the expanding knowledge of microbial genomes and physiology, and the increased specificity in function of genetic materials introduced through recombinant-DNA techniques compared with other forms of genetic manipulation, it may become easier to predict unintended effects of a particular modification. Molecular biological and biochemical techniques can also be used to analyse changes that occur at the level of transcription and translation that could lead to unintended effects.

21. The safety assessment of foods produced using recombinant-DNA micro-organisms involves methods to identify and detect such unintended effects, and procedures to evaluate their biological relevance and potential impact on food safety. A variety of

data and information is necessary to assess unintended effects, because no individual test can detect all possible unintended effects or identify, with certainty, those relevant to human health. These data and information, when considered in total, should provide assurance that the food is unlikely to have an adverse effect on human health. The assessment of unintended effects takes into account the biochemical and physiological characteristics of the micro-organism that are typically selected for improving strains for commercial food or beverage uses. These determinations provide a first screen for micro-organisms that exhibit unintended traits. Recombinant-DNA micro-organisms that pass this screen are subjected to safety assessment as described in Section 4.

Framework of food safety assessment

22. The safety assessment of a food produced using a recombinant-DNA micro-organism is based on determining the safety of using the micro-organism. It follows a stepwise process of addressing relevant factors, which include:

A. description of the recombinant-DNA micro-organism;
B. description of the recipient micro-organism and its use in food production;
C. description of the donor organism(s);
D. description of the genetic modification(s) including vector and construct;
E. characterization of the genetic modification(s);
F. safety assessment:
    a) expressed substances: assessment of potential toxicity and other traits related to pathogenicity,
    b) compositional analyses of key components,
    c) evaluation of metabolites,
    d) effects of food processing,
    e) assessment of immunological effects,
    f) assessment of viability and residence of micro-organisms in the human gastrointestinal tract,
    g) antibiotic resistance and gene transfer, and
    h) nutritional modification.

23. In certain cases, the characteristics of the micro-organisms and/or the foods produced/processed using these micro-organisms may necessitate generation of additional data and information to address issues that are unique to the micro-organisms and/or food products under review.

24. Experiments intended to develop data for safety assessments should be designed and conducted in accordance with sound scientific concepts and principles, as well as, where appropriate, good laboratory practice. Primary data should be made available to regulatory authorities upon request. Data should be obtained using sound scientific methods and analysed using appropriate statistical techniques. The sensitivity of all analytical methods should be documented.

25. The goal of each safety assessment is to provide assurance, in the light of the best available scientific knowledge, that the food will not cause harm when prepared or consumed according to its intended use, nor should the organism itself cause harm

when viable organisms remain in the food. Safety assessments should address the health aspects for the whole population, including immuno-compromised individuals, infants and the elderly. The expected end-point of such an assessment will be a conclusion regarding whether the new food and/or micro-organisms are as safe as the conventional counterparts, taking into account dietary impact of any changes in nutritional content or value. Where the micro-organism is likely to be viable upon ingestion, its safety should be compared with a conventional counterpart, taking into account residence of the recombinant-DNA micro-organism in the gastrointestinal tract, and where appropriate, interactions between it and the gastrointestinal flora of mammals (especially humans) and impacts of the recombinant-DNA micro-organism on the immune system. In essence, the outcome of the safety assessment process is to define the product under consideration in such a way as to enable risk managers to determine whether any measures are needed to protect the health of consumers and, if so, to make well-informed and appropriate decisions in this regard.

SECTION 4 – GENERAL CONSIDERATIONS

Description of the recombinant-DNA micro-organism

26. A description of the bacterial, yeast or fungal strain and the food being presented for safety assessment should be provided. This description should be sufficient to aid in understanding the nature of the organism or food produced using the organism being submitted for safety assessment. Recombinant-DNA micro-organisms used in food production or contained in food should be conserved as stock cultures with appropriate identification using molecular methods, and preferably, in established culture collections. This may facilitate the review of the original safety assessment. Such stock cultures should be made available to regulatory authorities upon request.

Description of the recipient micro-organism and its use in food production

27. A comprehensive description of the recipient micro-organism or micro-organism subjected to the modification should be provided. Recipient micro-organisms should have a history of safe use in food production or safe consumption in foods. Organisms that produce toxins, antibiotics or other substances that should not be present in food, or that bear genetic elements that could lead to genetic instability, antibiotic resistance, or that are likely to contain genes conferring functions associated with pathogenicity (i.e. also known as pathogenicity islands or virulence factors) should not be considered for use as recipients. The necessary data and information should include, but need not be restricted to:

A. identity: scientific name, common name or other name(s) used to reference the micro-organism, strain designation, information about the strain and its source, or accession numbers or other information from a recognized culture repository from which the organism or its antecedents may be obtained, if applicable, and information supporting its taxonomical assignment;
B. history of use and cultivation, known information about strain development (including isolation of mutations or antecedent strains used in strain construction); in particular, identifying traits that may adversely affect human health;

C. information on the genotype and phenotype of the recipient micro-organism relevant to its safety, including any known toxins, antibiotics, antibiotic resistance factors or other factors related to pathogenicity, or immunological impact, and information about the genetic stability of the micro-organism;
D. history of safe use in food production or safe consumption in food; and
E. information on the relevant production parameters used to culture the recipient micro-organism.

28. Relevant phenotypic and genotypic information should be provided not only for the recipient micro-organism, but also for related species and for any extra-chromosomal genetic elements that contribute to the functions of the recipient strain, particularly if the related species are used in foods or involved in pathogenic effects in humans or other animals. Information on the genetic stability of the recipient micro-organism should be considered including, as appropriate, the presence of mobile DNA elements, i.e. insertion sequences, transposons, plasmids and prophages.

29. The history of use may include information on how the recipient micro-organism is typically grown, transported and stored, quality assurance measures typically employed, including those to verify strain identity and production specifications for micro-organisms and foods, and whether these organisms remain viable in the processed food or are removed or rendered non-viable as a consequence of processing.

Description of the donor organism(s)

30. Information should be provided on the donor organism(s) and any intermediate organisms, when applicable, and, when relevant, related organisms. It is particularly important to determine if the donor or intermediate organism(s) or other closely related species naturally exhibit characteristics of pathogenicity or toxin production, or have other traits that affect human health. The description of the donor or intermediate organism(s) should include:

A. identity: scientific name, common name or other name(s) used to reference the organism, strain designation, information about the strain and its source, or accession numbers or other information from a recognized culture repository from which the organism or its antecedents may be obtained, if applicable, and information supporting its taxonomic assignment;
B. information about the organism or related organisms that concerns food safety;
C. information on the genotype and phenotype of the organism relevant to its safety including any known toxins, antibiotics, antibiotic resistance factors or other factors related to pathogenicity, or immunological impact; and
D. information on the past and present use, if any, in the food supply and exposure route(s) other than intended food use (e.g. possible presence as contaminants).

Description of the genetic modification(s) including vector and construct

31. Sufficient information should be provided on the genetic modification(s) to allow for the identification of all genetic material potentially delivered to or modified in the recipient micro-organism and to provide the necessary information for the analysis of

the data supporting the characterization of the DNA added to, inserted into, modified in or deleted from the microbial genome.

32. The description of the strain construction process should include:
   A. information on the specific method(s) used for genetic modification;
   B. information on the DNA used to modify the micro-organism, including the source (e.g. plant, microbial, viral, synthetic), identity and expected function in the recombinant-DNA micro-organism, and copy number for plasmids; and
   C. intermediate recipient organisms including the organisms (e.g. other bacteria or fungi) used to produce or process DNA prior to introduction into the final recipient organism.

33. Information should be provided on the DNA added, inserted, deleted or modified, including:
   A. the characterization of all genetic components, including marker genes, vector genes, regulatory and other elements affecting the function of the DNA;
   B. the size and identity;
   C. the location and orientation of the sequence in the final vector/construct; and
   D. the function.

Characterization of the genetic modification(s)

34. In order to provide clear understanding of the impact of the genetic modification on the composition and safety of foods produced using recombinant-DNA micro-organisms, a comprehensive molecular and biochemical characterization of the genetic modification should be carried out. To facilitate the safety assessment, the DNA to be inserted should preferably be limited to the sequences necessary to perform the intended functions.

35. Information should be provided on the DNA modifications in the recombinant DNA micro-organism; this should include:
   A. the characterization and description of the added, inserted, deleted or otherwise modified genetic materials, including plasmids or other carrier DNA used to transfer desired genetic sequences. This should include an analysis of the potential for mobilization of any plasmids or other genetic elements used, the locations of the added, inserted, deleted or otherwise modified genetic materials (site on a chromosomal or extra-chromosomal location); if located on a multicopy plasmid, the copy number of the plasmid;
   B. the number of insertion sites;
   C. the organization of the modified genetic material at each insertion site. including the copy number and sequence data of the inserted, modified or deleted material, plasmids or carrier DNA used to transfer the desired genetic sequences, and the surrounding sequences. This will enable the identification of any substances expressed as a consequence of the inserted, modified or deleted material;
   D. identification of any open reading frames within inserted DNA or created by the modifications to contiguous DNA in the chromosome or in a plasmid, including those that could result in fusion proteins; and

E. particular reference to any sequences known to encode, or to influence the expression of, potentially harmful functions.

36. Information should be provided on any expressed substances in the recombinant-DNA micro-organism; this should include:

A. the gene product(s) (e.g. a protein or an untranslated ribonucleic acid [RNA]) or other information such as analysis of transcripts or expression products to identify any new substances that may be present in the food;
B. the function of the gene product;
C. the phenotypic description of the new trait(s);
D. the level and site of expression (intracellular, periplasmic – for Gram-negative bacteria, organellar – in eukaryotic micro-organisms, secreted) in the micro-organism of the expressed gene product(s), and, when applicable, the levels of its metabolites in the organism;
E. the amount of the inserted gene product(s) if the function of the expressed sequence(s)/gene(s) is to alter the level of a specific endogenous messenger RNA (mRNA) or protein; and
F. the absence of a gene product, or alterations in metabolites related to gene products, if applicable to the intended function(s) of the genetic modification(s).

37. In addition, information should be provided:

A. to demonstrate whether the arrangement of the modified genetic material has been conserved[7] or whether significant rearrangements have occurred after introduction to the cell and propagation of the recombinant strain to the extent needed for its use(s) in food production, including those that may occur during its storage according to current techniques;
B. to demonstrate whether deliberate modifications made to the amino acid sequence of the expressed protein result in changes in its post-translational modification or affect sites critical for its structure or function;
C. to demonstrate whether the intended effect of the modification has been achieved and that all expressed traits are expressed and inherited in a manner that is stable for the extent of propagation needed for its use(s) in food production and is consistent with laws of inheritance. It may be necessary to examine the inheritance of the inserted or modified DNA or the expression of the corresponding RNA if the phenotypic characteristics cannot be measured directly;[8]
D. to demonstrate whether the newly expressed trait(s) is expressed as expected and targeted to the appropriate cellular location or is secreted in a manner and at levels that is consistent with the associated regulatory sequences driving the expression of the corresponding gene;

[7] Microbial genomes are more fluid than those of higher eukaryotes; that is, the organisms grow faster, adapt to changing environments, and are more prone to change. Chromosomal rearrangements are common. The general genetic plasticity of micro-organisms may affect recombinant DNA in micro-organisms and must be considered in evaluating the stability of recombinant DNA micro-organisms.

[8] Modified strains should be maintained in a manner to enable verification of the genetic stability.

E. to indicate whether there is any evidence to suggest that one or more genes in the recipient micro-organism have been affected by the modifications or the genetic exchange process; and
F. to confirm the identity and expression pattern of any new fusion proteins.

Safety assessment

38. The safety assessment of the modified micro-organism should be performed on a case-by-case basis depending on the nature and extent of the introduced changes. Conventional toxicology studies may not be considered necessary where the substance or a closely related substance has, taking into account its function and exposure, been consumed safely in food. In other cases, the use of appropriate conventional toxicology or other studies on the new substance may be necessary. Effects of the recombinant-DNA micro-organism on the food matrix should be considered as well. If the characterization of the food indicates that the available data are insufficient for a thorough safety assessment, properly designed animal or *in vitro* studies with the recombinant-DNA micro-organism and/or the food produced using it could be considered necessary.

Expressed substances: assessment of potential toxicity and other traits related to pathogenicity

39. When a substance is new to foods or food processing, the use of conventional toxicology studies or other applicable studies on the new substance will be necessary. This may require the isolation of the new substance from the recombinant-DNA micro-organism, the food product if the substance is secreted, or, if necessary, the synthesis or production of the substance from an alternative source, in which case the material should be shown to be structurally, functionally and biochemically equivalent to that produced in the recombinant-DNA micro-organism. Information on the anticipated exposure of consumers to the substance, and on the potential intake and dietary impact of the substance should be provided.

40. The safety assessment of the expressed substance should take into account its function and concentration in the food. The number of viable micro-organisms remaining in the food should be also determined and compared with a conventional counterpart. All quantitative measurements should be analysed using appropriate statistical techniques. Current dietary exposure and possible effects on population subgroups should also be considered.

- In the case of proteins, the assessment of potential toxicity should take into account the structure and function of the protein and should focus on amino acid sequence similarity between the protein and known protein toxins and antinutrients (e.g. protease inhibitors, siderophores) as well as stability to heat or processing and to degradation in appropriate representative gastric and intestinal model systems. Appropriate oral toxicity studies[9] may be carried out

[9] Guidelines for oral toxicity studies have been developed in international fora, for example the *OECD Guidelines for the Testing of Chemicals* issued by the Organisation for Economic Co-operation and Development.

in cases where the protein is present in the food, but is not closely similar to proteins that have been safely consumed in food and has not previously been consumed safely in food, and taking into account its biological function in micro-organisms, where known.

- Potential toxicity of non-protein substances that have not been safely consumed in food should be assessed on a case-by-case basis depending on the identity, concentration and biological function of the substance and dietary exposure. The type of studies to be performed may include evaluations of metabolism, toxicokinetics, chronic toxicity/ carcinogenicity, impact on reproductive function, and teratogenicity.

41. The newly expressed or altered properties should be shown to be unrelated to any characteristics of donor organisms that could be harmful to human health. Information should be provided to ensure that genes coding for known toxins or antinutrients present in the donor organisms are not transferred to recombinant-DNA micro-organisms that do not normally express those toxic or antinutritious characteristics.

- Additional *in vivo* or *in vitro* studies may be needed on a case-by-case basis to assess the toxicity of expressed substances, taking into account the potential accumulation of any substances, toxic metabolites or antibiotics that might result from the genetic modification.

Compositional analyses of key components

42. Analyses of concentrations of key components[10] of foods produced by recombinant-DNA micro-organisms should be compared with an equivalent analysis of a conventional counterpart produced under the same conditions. The statistical significance of any observed differences should be assessed in the context of the range of natural variations for that parameter to determine its biological significance. Ideally, the comparator(s) used in this assessment should be food produced using the near isogenic parent strain. The purpose of this comparison, in conjunction with an exposure assessment as necessary, is to establish that substances that can affect the safety of the food have not been altered in a manner that would have an adverse impact on human health.

Evaluation of metabolites

43. Some recombinant-DNA micro-organisms may be modified in a manner that could result in new or altered levels of various metabolites in foods produced using these organisms. Where altered metabolite levels are identified in foods, consideration should be given to the potential impacts on human health, using conventional procedures for

[10] Key nutrients or key antinutrients are those components in a particular food that may have a substantial impact in the overall diet. They may be major nutritional constituents (fats, proteins, carbohydrates), enzyme inhibitors as antinutrients, or minor compounds (minerals, vitamins). Key toxicants are those toxicologically significant compounds known to be produced by the micro-organism, such as those compounds whose toxic potency and level may be significant to health. Micro-organisms traditionally used in food processing are not usually known to produce such compounds under production conditions.

establishing the safety of such metabolites (e.g. procedures for assessing the human safety of chemicals in foods).

44. New or altered levels of metabolites produced by a recombinant-DNA micro-organism may change the population of micro-organisms in mixed culture, potentially increasing the risk for growth of harmful organisms or accumulation of harmful substances. Possible effects of genetic modification of a micro-organism on other micro-organisms should be assessed when a mixed culture of micro-organisms is used for food processing, such as for production of natural cheese, miso and soy sauce.

Effects of food processing

45. The potential effects of food processing, including home preparation, on foods produced using recombinant-DNA micro-organisms should also be considered. For example, alterations could occur in the heat stability of an endogenous toxicant or the bioavailability of an important nutrient after processing. Therefore, information should be provided, describing the processing conditions used in the production of a food. For example, in the case of yoghurt, information should be provided on the growth of the organism and culture conditions.

Assessment of immunological effects

46. When the protein(s) resulting from an inserted gene is present in the food, it should be assessed for its potential to cause allergy. The likelihood that individuals may already be sensitive to the protein and whether a protein new to the food supply will induce allergic reactions should be considered. A detailed presentation of issues to be considered is presented in the Annex to this Guideline.

47. Genes derived from known allergenic sources should be assumed to encode an allergen and be avoided unless scientific evidence demonstrates otherwise. The transfer of genes from organisms known to elicit gluten-sensitive enteropathy in sensitive individuals should be avoided unless it is documented that the transferred gene does not code for an allergen or for a protein involved in gluten-sensitive enteropathy.

48. Recombinant-DNA micro-organisms that remain viable in foods may interact with the immune system in the gastrointestinal tract. Closer examination of these interactions will depend on the types of differences between the recombinant-DNA micro-organism and its conventional counterpart.

Assessment of viability and residence of micro-organisms in the human gastrointestinal tract

49. In some foods produced using recombinant-DNA micro-organisms, ingestion of these micro-organisms and their residence[11] may have an impact on the human intestinal

[11] Permanent life-long colonization by ingested micro-organisms is rare. Some orally administered micro-organisms have been recovered in faeces or in the colonic mucosa weeks after feeding ceased. Whether the genetically modified micro-organism is established in the gastrointestinal tract or not, the possibility remains that it might influence the microflora or the mammalian host (Joint FAO/WHO Expert Consultation on Foods Derived from Biotechnology, *Safety assessment of foods derived from genetically modified micro-organism,* 24–28 September 2001, Geneva, Switzerland).

tract. The need for further testing of such micro-organisms should be based on the presence of their conventional counterpart in foods, and the nature of the intended and unintended effects of genetic modifications. If processing of the final food product eliminates viable micro-organisms (by heat treatment in baking bread, for example) or if accumulations of end-products toxic to the micro-organism (such as alcohol or acids) eliminate viability, then viability and residence of micro-organisms in the alimentary system need no examination.

50. For applications in which recombinant-DNA micro-organisms used in production remain viable in the final food product (e.g. organisms in some dairy products), it may be desirable to demonstrate the viability (or residence time) of the micro-organism alone and within the respective food matrix in the digestive tract, and the impact on the intestinal microflora in appropriate systems. The nature of intended and unintended effects of genetic modification and the degree of differences from the conventional counterpart will determine the extent of such testing.

Antibiotic resistance and gene transfer

51. In general, traditional strains of micro-organisms developed for food processing uses have not been assessed for antibiotic resistance. Many micro-organisms used in food production possess intrinsic resistance to specific antibiotics. Such properties need not exclude such strains from consideration as recipients in constructing recombinant-DNA micro-organisms. However, strains in which antibiotic resistance is encoded by transmissible genetic elements should not be used where such strains or these genetic elements are present in the final food. Any indication of the presence of plasmids, transposons and integrons containing such resistance genes should be specifically addressed.

52. Alternative technologies, demonstrated to be safe, that do not rely on antibiotic resistance marker genes in viable micro-organisms present in foods should be used for selection purposes in recombinant-DNA micro-organisms. In general, use of antibiotic resistance markers for constructing intermediate strains should pose no significant hazards that would exclude the use of the ultimate strains in food production, provided that the antibiotic resistance marker genes have been removed from the final construct.

53. Transfer of plasmids and genes between the resident intestinal microflora and ingested recombinant-DNA micro-organisms may occur. The possibility and consequences of gene transfer from recombinant-DNA micro-organisms and food products produced by recombinant-DNA micro-organisms to gut micro-organisms or human cells should also be considered. Transferred DNA would be unlikely to be maintained in the absence of selective pressure. Nevertheless, the possibility of such events cannot be completely discounted.

54. In order to minimize the possibility of gene transfer, the following steps should be considered:

A. Chromosomal integration of the inserted genetic material may be preferable to localization on a plasmid.
B. Where the recombinant-DNA micro-organism will remain viable in the gastrointestinal tract, genes that could provide a selective advantage to recipient organisms to which the genetic material is unintentionally transferred should be avoided in the genetic construct.
C. Sequences that mediate integration into other genomes should be avoided in constructing the introduced genetic material.

Nutritional modification

55. The assessment of possible compositional changes to key nutrients, which should be conducted for all foods produced using recombinant-DNA micro-organisms, has already been addressed under "Compositional analyses of key components". If such nutritional modifications have been implemented, the food 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.

56. Information about the known patterns of use and consumption of a food and its derivatives should be used to estimate the likely intake of the food produced using the recombinant-DNA micro-organism. The expected intake of the food should be used to assess the nutritional implications of the altered nutrient profile both at customary and maximal levels of consumption. Basing the estimate on the highest likely consumption provides assurance that the potential for any undesirable nutritional effects will be detected. Attention should be paid to the particular physiological characteristics and metabolic requirements of specific population groups, such as infants, children, pregnant and lactating women, the elderly and those with chronic diseases or compromised immune systems. Based on the analysis of nutritional impacts and the dietary needs of specific population subgroups, additional nutritional assessments may be necessary. It is also important to ascertain to what extent the modified nutrient is bioavailable and remains stable with time, processing and storage.

57. The use of modern biotechnology to change nutrient levels in foods produced using micro-organisms could result in broad changes to the nutrient profile. The intended modification in the micro-organism could alter the overall nutrient 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.

58. When the modification results in a food product with a composition that is significantly different from its conventional counterpart, it may be appropriate to use additional conventional foods or food components (i.e. foods whose nutritional composition is closer to that of the food produced using the recombinant-DNA micro-organism) as appropriate comparators to assess the nutritional impact of the food.

59. Some foods may require additional testing. For example, animal-feeding studies may be warranted for foods produced using recombinant-DNA micro-organisms if changes

in the bioavailability of nutrients are expected or if the composition is not comparable with conventional foods. In addition, foods designed for health benefits may require an assessment beyond the scope of this Guideline such as specific nutritional, toxicological or other appropriate studies. If the characterization of the food indicates that the available data are insufficient for a thorough safety assessment, properly designed animal studies could be requested on the whole food.

Review of safety assessments

60. The goal of the safety assessment is a conclusion as to whether the food produced using a recombinant-DNA micro-organism is as safe as the conventional counterpart, taking into account dietary impact of any changes in nutritional content or value. Nevertheless, the safety assessment should be reviewed in the light of new scientific information that calls into question the conclusions of the original safety assessment.

ANNEX

ASSESSMENT OF POSSIBLE ALLERGENICITY

SECTION 1 – INTRODUCTION

1. All newly expressed proteins[12] produced by recombinant-DNA micro-organisms that could be present in the final food should be assessed for their potential to cause allergic reactions. This should include consideration of whether a newly expressed protein is one to which certain individuals may already be sensitive as well as whether a protein new to the food supply is likely to induce allergic reactions in some individuals.

2. At present, there is no definitive test that can be relied upon to predict allergic response in humans to a newly expressed protein. Therefore, it is recommended that an integrated, stepwise, case-by-case approach, as described below, be used in the assessment of possible allergenicity of newly expressed proteins. This approach takes into account the evidence derived from several types of information and data as no single criterion is sufficiently predictive.

3. The end-point of the assessment is a conclusion as to the likelihood of the protein being a food allergen.

SECTION 2 – ASSESSMENT STRATEGY

4. The initial steps in assessing possible allergenicity of any newly expressed proteins are the determination of: the source of the introduced protein; any significant similarity between the amino acid sequence of the protein and that of known allergens; and its structural properties, including, but not limited to, its susceptibility to enzymatic degradation, heat stability and/or acid and enzymatic treatment.

5. As there is no single test that can predict the likely human immunoglobulin E (IgE) response to oral exposure, the first step to characterize newly expressed proteins should be the comparison of the amino acid sequence and certain physicochemical characteristics of the newly expressed protein with those of established allergens in a weight of evidence approach. This will require the isolation of any newly expressed proteins produced by recombinant-DNA micro-organisms, or the synthesis or production of the substance from an alternative source, in which case the material should be shown to be structurally, functionally and biochemically equivalent to that produced by recombinant-DNA micro-organisms. Particular attention should be given to the choice of the expression host, as post-translational modifications allowed by different

[12] This assessment strategy is not applicable for assessing whether newly expressed proteins are capable of inducing gluten-sensitive or other enteropathies. The issue of enteropathies is addressed in paragraph 47 of the *Guideline for the conduct of food safety assessment of foods produced using recombinant-DNA micro-organisms* (CAC/GL 46-2003). In addition, the strategy is not applicable to the evaluation of foods where gene products are down regulated for hypoallergenic purposes.

hosts (i.e. eukaryotic vs prokaryotic systems) may have an impact on the allergenic potential of the protein.

6. It is important to establish whether the source is known to cause allergic reactions. Genes derived from known allergenic sources should be assumed to encode an allergen unless scientific evidence demonstrates otherwise.

SECTION 3 – INITIAL ASSESSMENT

Section 3.1 – Source of the protein

7. As part of the data supporting the safety of foods produced using recombinant-DNA micro-organisms, information should describe any reports of allergenicity associated with the donor organism. Allergenic sources of genes would be defined as those organisms for which reasonable evidence of IgE mediated oral, respiratory or contact allergy is available. Knowledge of the source of the introduced protein allows the identification of tools and relevant data to be considered in the allergenicity assessment. These include: the availability of sera for screening purposes; documented type, severity and frequency of allergic reactions; structural characteristics and amino acid sequence; physicochemical and immunological properties (when available) of known allergenic proteins from that source.

Section 3.2 – Amino acid sequence homology

8. The purpose of a sequence homology comparison is to assess the extent to which a newly expressed protein is similar in structure to a known allergen. This information may suggest whether that protein has an allergenic potential. Sequence homology searches comparing the structure of all newly expressed proteins with all known allergens should be done. Searches should be conducted using various algorithms such as FASTA or BLASTP to predict overall structural similarities. Strategies such as stepwise contiguous identical amino acid segment searches may also be performed for identifying sequences that may represent linear epitopes. The size of the contiguous amino acid search should be based on a scientifically justified rationale in order to minimize the potential for false negative or false positive results.[13] Validated search and evaluation procedures should be used in order to produce biologically meaningful results.

9. IgE cross-reactivity between the newly expressed protein and a known allergen should be considered a possibility when there is more than 35 percent identity in a segment of 80 or more amino acids (FAO/WHO 2001) or other scientifically justified criteria. All the information resulting from the sequence homology comparison between the newly expressed protein and known allergens should be reported to allow a case-by-case scientifically-based evaluation.

[13] It is recognized that the 2001 FAO/WHO consultation suggested moving from 8 to 6 identical amino acid segment searches. The smaller the peptide sequence used in the stepwise comparison, the greater the likelihood of identifying false positives; inversely, the larger the peptide sequence used, the greater the likelihood of false negatives, thereby reducing the utility of the comparison.

10. Sequence homology searches have certain limitations. In particular, comparisons are limited to the sequences of known allergens in publicly available databases and the scientific literature. There are also limitations in the ability of such comparisons to detect non-contiguous epitopes capable of binding themselves specifically with IgE antibodies.

11. A negative sequence homology result indicates that a newly expressed protein is not a known allergen and is unlikely to be cross-reactive to known allergens. A result indicating absence of significant sequence homology should be considered along with the other data outlined under this strategy in assessing the allergenic potential of newly expressed proteins. Further studies should be conducted as appropriate (see also Sections 4 and 5). A positive sequence homology result indicates that the newly expressed protein is likely to be allergenic. If the product is to be considered further, it should be assessed using serum from individuals sensitized to the identified allergenic source.

Section 3.3 – Pepsin resistance

12. Resistance to pepsin digestion has been observed in several food allergens; thus, a correlation exists between resistance to digestion by pepsin and allergenic potential.[14] Therefore, the resistance of a protein to degradation in the presence of pepsin under appropriate conditions indicates that further analysis should be conducted to determine the likelihood of the newly expressed protein being allergenic. The establishment of a consistent and well-validated pepsin degradation protocol may enhance the utility of this method. However, it should be taken into account that a lack of resistance to pepsin does not exclude that the newly expressed protein can be a relevant allergen.

13. Although the pepsin resistance protocol is strongly recommended, it is recognized that other enzyme susceptibility protocols exist. Alternative protocols may be used where adequate justification is provided.[15]

SECTION 4 – SPECIFIC SERUM SCREENING

14. For those proteins that originate from a source known to be allergenic, or have sequence homology with a known allergen, testing in immunological assays should be performed where sera are available. Sera from individuals with a clinically validated allergy to the source of the protein can be used to test the specific binding to IgE class antibodies of the protein in *in vitro* assays. A critical issue for testing will be the availability of human sera from sufficient numbers of individuals.[16] In addition, the

[14] The method outlined in *The United States Pharmacopoeia* (1995) was used in the establishment of the correlation (Astwood *et al.,* 1996).

[15] Reference to Joint FAO/WHO Expert Consultation (2001).

[16] According to the report of the Joint FAO/WHO Expert Consultation on allergenicity of foods derived from biotechnology (22–25 January 2001, Rome) a minimum of eight relevant sera is required in order to achieve a 99-percent certainty that the new protein is not an allergen in the case of a major allergen. Similarly, a minimum of 24 relevant sera is required to achieve the same level of certainty in the case of a minor allergen. It is recognized that these quantities of sera may not be available for testing purposes.

quality of the sera and the assay procedure need to be standardized to produce a valid test result. For proteins from sources not known to be allergenic and which do not exhibit sequence homology to a known allergen, targeted serum screening may be considered where such tests are available as described in paragraph 17.

15. In the case of a newly expressed protein derived from a known allergenic source, a negative result in *in vitro* immunoassays may not be considered sufficient, but should prompt additional testing, such as the possible use of skin test and *ex vivo* protocols.[17] A positive result in such tests would indicate a potential allergen.

SECTION 5 – OTHER CONSIDERATIONS

16. The absolute exposure to the newly expressed protein and the effects of relevant food processing will contribute towards an overall conclusion about the potential for human health risk. In this regard, the nature of the food product intended for consumption should be taken into consideration in determining the types of processing that would be applied and its effects on the presence of the protein in the final food product.

17. As scientific knowledge and technology evolve, other methods and tools may be considered in assessing the allergenicity potential of newly expressed proteins as part of the assessment strategy. These methods should be scientifically sound and may include: targeted serum screening (i.e. the assessment of binding to IgE in sera of individuals with clinically validated allergic responses to broadly related categories of foods); the development of international serum banks; use of animal models; and examination of newly expressed proteins for T-cell epitopes and structural motifs associated with allergens.

[17] Reference to the joint FAO/WHO Expert Consultation (2001) on description of *ex vivo*.

GUIDELINE FOR THE CONDUCT OF FOOD SAFETY ASSESSMENT OF FOODS DERIVED FROM RECOMBINANT-DNA ANIMALS

CAC/GL 68-2008

SECTION 1 – SCOPE

1. This Guideline supports the *Principles for the risk analysis of foods derived from modern biotechnology* (CAC/GL 44-2003). It addresses safety and nutritional aspects of foods consisting of, or derived from, animals that have a history of safe use as sources of food, and that have been modified by modern biotechnology to exhibit new or altered expression of traits.[1]

2. The development, raising and use of animals for human purposes and, in particular, for use for food raise a variety of issues beyond food safety. Without prejudice to their legitimacy or importance, or to whether or how the use of recombinant deoxyribonucleic acid (DNA) methods in developing animals for food use might affect those issues, this Guideline addresses only food safety and nutritional issues. Therefore, it does not address:
   - animal welfare;
   - ethical, moral and socio-economic aspects;
   - environmental risks related to the environmental release of recombinant-DNA animals used in food production;
   - the safety of recombinant-DNA animals used as feed, or the safety of animals fed with feed derived from recombinant-DNA animals, plants and micro-organisms.

3. The Codex Principles of risk analysis, particularly those for risk assessment, are primarily intended to apply to discrete chemical entities, such as food additives and pesticide residues, or a specific chemical or microbial contaminant, that have identifiable hazards and risks; they are not intended to apply to whole foods as such. Indeed, few foods, whatever their origin, have been assessed scientifically in a manner that would fully characterize all risk associated with the food. Further, many foods contain substances that would probably be found harmful if subjected to conventional approaches to safety testing. Thus, a more focused approach is required where the safety of a whole food is being considered.

4. This approach is based on the principle that the safety of foods derived from new animal lines, including recombinant-DNA animals, is assessed relative to the conventional counterpart having a history of safe use, taking into account both intended and unintended effects. Rather than trying to identify every hazard associated with a

[1] This Guideline was developed primarily for animals bearing heritable recombinant-DNA constructs.

particular food, the intention is to identify new or altered hazards relative to the conventional counterpart.

5. This safety assessment approach falls within the risk assessment framework as discussed in Section 3 of the *Principles for the risk analysis of foods derived from modern biotechnology* (CAC/GL 44-2003). If a new or altered hazard, nutritional or other food safety concern is identified by the safety assessment, the risk associated with it would first be assessed to determine its relevance to human health. Following the safety assessment and, if necessary, further risk assessment, the food would be subjected to risk management considerations in accordance with the *Principles for the risk analysis of foods derived from modern biotechnology* (CAC/GL 44-2003) before being considered for commercial distribution.

6. Risk management measures such as post-market monitoring of consumer health effects may assist the risk assessment process. These are discussed in paragraph 20 of the *Principles for the risk analysis of foods derived from modern biotechnology* (CAC/GL 44-2003).

7. The Guideline describes the recommended approach for the food safety assessment of foods derived from recombinant-DNA animals where a conventional counterpart exists, and identifies the data and information that are generally applicable to making such assessments.[2] In assessing the safety of food from recombinant-DNA animals, the approach should take into account all of the following:
   A. the nature of the recombinant-DNA construct and its expression product(s), if any;
   B. the health status of the recombinant-DNA animal; and
   C. the composition of foods produced from recombinant-DNA animals, including key nutrients.

   While this Guideline is designed for foods derived from recombinant-DNA animals, the approach described could, in general, be applied to foods derived from animals that have been altered by other techniques.[3]

8. A diverse range of animals is used as food or for food production (e.g. mammals, birds, finfish and shellfish) and may be modified using *in vitro* nucleic acid techniques. Because of the combined impacts of their genetic diversity, husbandry and conditions under which they are raised or harvested, assessment of food safety must be considered on a case-by-case basis, with due regard to the framework presented in this Guideline.

[2] The approach to the safety assessment of foods derived from recombinant-DNA animals was first discussed at the 1991 Joint FAO/WHO Consultation on strategies for assessing the safety of foods produced by biotechnology. Further elaboration of the recommended approach was undertaken at the 2003 Joint FAO/WHO Expert Consultation on the safety assessment of foods derived from genetically modified animals, including fish.

[3] The food safety assessment of foods derived from animals bearing non-heritable constructs may require additional specific consideration, e.g. regarding hazards identified in the 2007 Joint FAO/WHO Expert Consultation on the safety assessment of foods derived from recombinant-DNA animals.

SECTION 2 – DEFINITIONS

9. The definitions below apply to this Guideline:

Recombinant-DNA animal means an animal in which the genetic material has been changed through *in vitro* nucleic acid techniques, including recombinant deoxyribonucleic acid (DNA) and direct injection of nucleic acid into cells or organelles.

Conventional counterpart means an animal breed with a known history of safe use as food from which the recombinant-DNA animal line was derived, as well as the breeding partners used in generating the animals ultimately used as food, and/or food derived from such animals.[4]

SECTION 3 – INTRODUCTION TO FOOD SAFETY ASSESSMENT

10. Traditionally, food products derived from animals developed through conventional breeding or obtained from wild species have not been systematically subjected to extensive chemical, toxicological or nutritional evaluation prior to marketing. Thus, although new breeds of animals are often evaluated by breeders for phenotypic characteristics, they are not subjected to the rigorous and extensive food safety testing procedures, including validated toxicity studies in test animals, that are typical of chemicals such as food additives or contaminants that may be present in food. Instead, food derived from an animal of known and acceptable health status has generally been considered suitable for human consumption.

11. The use of animal models for assessing toxicological end-points is a major element in the risk assessment of many compounds, such as pesticides. However, in most cases, the substance to be tested is well characterized, of known purity, of no particular nutritional value, and human exposure to it is generally low. Therefore, it is relatively straightforward to feed such compounds to test animals at a range of doses some several orders of magnitude greater than the expected human exposure levels in order to identify any potential adverse health effects of importance to humans. In this way, it is possible, in most cases, to estimate levels of exposure at which adverse effects are not observed and to set safe intake levels by the application of appropriate safety factors.

12. Studies using test animals cannot readily be applied to testing the risks associated with whole foods, which are complex mixtures of compounds and often characterized by a wide variation in composition and nutritional value. Owing to their bulk and effect on satiety, they can usually only be fed to test animals at low multiples of the amounts that might be present in the human diet. In addition, a key factor to consider in conducting animal studies on foods is the nutritional value and balance of the diets used, in order to avoid the induction of adverse effects that are not related directly to the

[4] It is recognized that for the foreseeable future, foods derived from modern biotechnology will not be used as conventional counterparts.

material itself. Detecting any potential adverse effects and relating these conclusively to an individual characteristic of the food can, therefore, be extremely difficult. If the characterization of the food indicates that the available data are insufficient for a thorough safety assessment, properly designed studies using test animals could be requested on the whole food. Another consideration in deciding the need for studies with test animals is whether it is appropriate to subject test animals to such a study if it is unlikely to give rise to meaningful information.

13. Owing to the difficulties of applying traditional toxicological testing and risk assessment procedures to whole foods, and based on the experience of assessing the safety of whole foods, a more focused approach is required for the safety assessment of food derived from animals, including recombinant-DNA animals. This has been addressed by the development of a multidisciplinary approach for assessing safety that takes into account both intended and unintended changes that may occur in the animal or in the food products derived from it, using the concept of substantial equivalence.

14. The concept of substantial equivalence is a key step in the safety assessment process. However, it is not a safety assessment in itself; rather, it represents the starting point that is used to structure the safety assessment of a new food relative to its conventional counterpart. This concept is used to identify similarities and differences between the new food and its conventional counterpart.[5] It aids in the identification of potential food safety and nutritional issues and is considered the most appropriate strategy to date for safety assessment of foods derived from recombinant-DNA animals. The safety assessment carried out in this way does not imply absolute safety of the new product; rather, it focuses on assessing the safety of any identified differences so that the safety of the new product can be considered relative to its conventional counterpart.

Unintended effects

15. In achieving the objective of conferring a specific trait (intended effect) to an animal by the insertion of defined DNA sequences, additional traits could, in some cases, be acquired or existing traits could be lost or modified (unintended effects). The potential occurrence of unintended effects is not restricted to the use of *in vitro* nucleic acid techniques. Rather, it is an inherent and general phenomenon that can also occur in conventional breeding as well in association with the use of assisted reproductive technologies currently in use. Unintended effects may be deleterious, beneficial or neutral with respect to the health of the animal or the safety of the foods derived from the animal. Unintended effects in recombinant-DNA animal may also arise through the insertion of DNA sequences and/or they may arise through subsequent conventional breeding of the recombinant-DNA animal. Safety assessment should include data and information to reduce the possibility that a food derived from a recombinant-DNA animal would have an unexpected, adverse effect on human health.

[5] The concept of substantial equivalence as described in the report of the 2000 Joint FAO/WHO Expert Consultation (*Safety aspects of genetically modified foods of plant origin*, WHO/SDE/PHE/FOS/00.6, WHO, Geneva, 2000). The concept of substantial equivalence was further considered in the context of comparative safety assessment at the 2003 Joint FAO/WHO Expert Consultation on the Safety Assessment of Foods Derived from Genetically Modified Animals, including Fish.

16. Unintended effects can result from the random insertion of DNA sequences into the animal genome, which may cause disruption or silencing of existing genes, activation of silent genes, or modifications in the expression of existing genes. Unintended effects may also result in the formation of new or changed patterns of metabolites.

17. Unintended effects caused by *in vitro* nucleic acid techniques may be subdivided into two groups: those that are "predictable", and those that are "unexpected". Many unintended effects are largely predictable based on knowledge of the inserted trait and its metabolic connections or of the site of insertion. As knowledge of animal genomes grows and familiarity with *in vitro* nucleic acid techniques increases, it may become easier to predict unintended effects of a particular modification. For example, homologous recombination, where appropriate, allows precise gene placement and so may reduce the occurrence of unintended effects associated with random integration. Molecular biological and biochemical techniques can also be used to analyse changes that occur at the level of transcription and translation that could lead to unintended effects. These should all be considered on a case-by-case basis.

18. The safety assessment of food derived from recombinant-DNA animals involves methods to identify and detect such unintended effects and procedures to evaluate their biological relevance and potential impact on food safety. A variety of data and information is necessary in order to assess unintended effects, because no individual test can detect all possible unintended effects or identify, with certainty, those relevant to human health. These data and information, when considered in total, provide assurance that the food is unlikely to have an adverse effect on human health. The assessment of unintended effects takes into account the phenotypic characteristics of the animal that are typically monitored by breeders during animal production stock development and improvement. These assessments provide a first screen for recombinant-DNA animals exhibiting unintended traits. Recombinant-DNA animals that pass this screen are subjected to safety assessment as described in Sections 4 and 5.

Framework of food safety assessment

19. The safety assessment follows a stepwise process of addressing relevant factors that include:

A. general description of the recombinant-DNA animal;
B. description of the recipient animal prior to the modification[6] and its use as food or for food production;
C. description of the donor organism or other source(s) of the introduced recombinant-DNA;
D. description of the genetic modification(s) including the construct(s) used to introduce the recombinant-DNA;

[6] Not to be confused with a surrogate dam.

E. description of the methods used to produce the initial recombinant-DNA animal[7] and the processes to produce the recombinant-DNA animal ultimately used as food or for food production;
F. characterization of the genetic modification(s) in the recombinant-DNA animal ultimately used as food or for food production;
G. safety assessment:
   a) health status of the recombinant-DNA animal,
   b) expressed substances (non-nucleic acid substances),
   c) compositional analyses of key components,
   d) food storage and processing, and
   e) intended nutritional modification;
H. other considerations.

20. In certain cases, the characteristics of the food may necessitate additional data and information to address issues that are unique to the product under review.

21. Experiments intended to develop data for safety assessment should be designed and conducted in accordance with sound scientific concepts and principles as well as, where appropriate, good laboratory practice. Primary data should be made available to regulatory authorities upon request. Data should be obtained using sound scientific methods and analysed using appropriate statistical techniques. Analytical methods should be documented.[8]

22. The goal of each safety assessment is to provide assurance, in the light of the best available scientific knowledge, that the food does not cause harm when prepared, used and/or eaten according to its intended use. Safety assessments should address the health aspects for the whole population, including immunocompromised individuals, infants, the elderly and individuals with food hypersensitivities. The expected end-point of such an assessment will be a conclusion regarding whether the new food is as safe as the conventional counterpart taking into account dietary impact of any changes in nutritional content or value. Therefore, in essence, the outcome of the safety assessment process is to define the product under consideration in such a way as to enable risk managers to determine whether any measures are needed to protect the health of consumers and, if so, to make well-informed and appropriate decisions in this regard.

SECTION 4 – GENERAL CONSIDERATIONS

General description of the recombinant-DNA animal

23. A description of the recombinant-DNA animal being presented for safety assessment should be provided. This description should identify the introduced recombinant-DNA, the method by which the recombinant-DNA is introduced to the recipient animal and

[7] First animal produced as a result of introducing the recombinant-DNA construct. Sometimes referred to as the founder animal.

[8] Reference is made to the "General criteria for the selection of methods of analysis" in the *Codex Alimentarius Commission Procedural Manual.*

the recombinant-DNA animal ultimately used as food or for food production, as well as the purpose of the modification. The potential risk of introducing pathogenic elements (e.g. elements responsible for transmissible spongiform encephalopathies and other infectious disease) originating from biological materials used as sources or during the production should be considered. The description should be sufficient to aid in understanding the nature and types of food being submitted for safety assessment.

Description of the recipient animal prior to the modification and its use as food or for food production

24. A comprehensive description of the recipient animal prior to the modification should be provided. The necessary data and information should include, but need not be restricted to:

A. common or usual name, scientific name, and taxonomic classification;
B. history of development through breeding, in particular identifying traits that may adversely affect human health;
C. information on the genotype and phenotype of the animal relevant to its safety, including any known toxicity or allergenicity, symbiosis with toxin-producing organisms, potential for colonization by human pathogens;
D. information on the effect of feed, exercise and growth environment on food products; and
E. history of safe use as food or for food production.

25. Relevant phenotypic information should be provided not only for the recipient animal prior to the modification, but also for related lines and for animals that have made or may make a significant contribution to the genetic background of the recipient animal prior to the modification, if applicable.

26. The history of use may include information on how the animals breed and grow, how their food products are obtained (e.g. harvest, slaughter, milking), and the conditions under which those food products are made available to the consumer (e.g. storage, transport, processing). The extent to which the food products provide important nutritional components to particular subgroups of the population, and what important macronutrients or micronutrients they contribute to the diet should also be considered.

Description of the donor organism or other source(s) of the introduced recombinant-DNA

27. Information should be provided:

A. on whether the recombinant-DNA was synthesized and it is not from a known natural source;
B. if derived from another organism:
   i) usual or common name of that organism;
   ii) scientific name;
   iii) taxonomic classification;
   iv) information about the natural history as concerns food safety;
   v) information on naturally occurring toxins, and allergens;

vi) for micro-organisms, additional information on pathogenicity (to humans or the animal) and the relationship to known human or animal pathogens;
vii) for donors of animal or viral origin, information on the source material (e.g. cell culture) that has been used, and its origins; and
viii) information on the past and present use, if any, in the food supply and exposure route(s) other than the intended food use (e.g. possible presence of contaminants).

It is particularly important to determine whether the recombinant-DNA sequences impart pathogenicity or toxin production, or have other traits that affect human health (e.g. allergenicity).

Description of the genetic modification(s) including the construct(s) used to introduce the recombinant-DNA

28. Sufficient information should be provided on the genetic modification to allow for the identification of all genetic material potentially delivered to the recipient animal and to provide the necessary information for the analysis of the data supporting the characterization of the DNA inserted into the recombinant-DNA animal ultimately used as food or for food production.

29. The description of the process of introducing and incorporating (if appropriate) the recombinant-DNA into the recipient animal should include:
A. information on the specific methodology used for the transformation;
B. information, if applicable, on the DNA used to modify the animal (e.g. genes coding for proteins used for packaging vectors), including the source, identity and expected function in the animal:
– if viral vectors or known zoonotic organisms have been used, information on their natural hosts, target organs, transmission mode, pathogenicity, and potential for recombination with endogenous or exogenous pathogens; and
C. intermediate host organisms including the organisms (e.g. bacteria) used to produce or process DNA for producing the initial recombinant DNA animal.

30. Information should be provided on the DNA to be introduced, including:
A. the primary DNA sequence if the recombinant-DNA was synthesized and it is not from a known natural source;
B. the characterization of all the genetic components including marker genes, regulatory and other elements affecting the expression and function of the DNA;
C. the size and identity;
D. the location and orientation of the sequence in the final vector/construct; and
E. the function.

Description of the methods used to produce the initial recombinant-DNA animal and the processes to produce the recombinant-DNA animal ultimately used as food or for food production

31. Information should be provided on the various techniques and processes that are used to introduce the recombinant-DNA to obtain the initial recombinant-DNA animal.

Examples of possible techniques may include transformation of gametes, microinjection of early embryos, nuclear transfer of transgenic cells.

32. A description of the methods used to demonstrate heritability should be provided, including descriptions of how heritability is attained (e.g. breeding mosaic animals to obtain true germ-cell transmissible insertions).

33. Although initial recombinant-DNA animals are generally not intended to be used as food or for food production, knowledge of the method to generate these animals may be useful in hazard identification.

34. Information should also be provided on how the initial recombinant-DNA animal leads to the production of the animal ultimately used as food or for food production. This information should, if applicable, include information on the breeding partners or surrogate dams, including genotype and phenotype, husbandry, and conditions under which they are raised or harvested.

35. The history of use of food products from the animals used to generate the animals ultimately used for food production from the initial recombinant-DNA animal (e.g. breeding partners, surrogate dams) may include information on how the animals breed and grow, how their food products are obtained (e.g. harvest, slaughter, milking), and the conditions under which those food products are made available to consumers (e.g. storage, transport, processing).

Characterization of the genetic modification(s) in the recombinant-DNA animal ultimately used as food or for food production

36. In order to provide clear understanding of the impact on the composition and safety of foods derived from recombinant-DNA animals, a comprehensive molecular and biochemical characterization of the genetic modification should be carried out.

37. Information should be provided on the DNA insertions into the animal genome; this should include:

A. the characterization and description of the inserted genetic materials. This should include an analysis of the potential for mobilization or recombination of any construct material used;
B. the number of insertion sites;
C. the organization of the inserted genetic material at each insertion site including copy number and sequence data of the inserted material and of the surrounding region, sufficient to identify any substances expressed as a consequence of the inserted material, or, where scientifically more appropriate, other information such as analysis of transcripts or expression products to identify any new substances that may be present in the food; and
D. identification of any open reading frames within the inserted DNA or created by insertion with contiguous animal genomic DNA, including those that could result in fusion proteins.

38. Information should be provided on any newly expressed substances in the recombinant-DNA animal; this should include:

A. the gene product(s) (e.g. a protein or an untranslated ribonucleic acid [RNA]) or other information such as analysis of transcripts or expression products to identify any new substances that may be present in the food;
B. the function of the gene product(s);
C. the phenotypic description of the new trait(s);
D. the level and site of expression in the animal of the expressed gene product(s), and the levels of its metabolites in the food; and
E. where possible, the amount of the target gene product(s) if the function of the expressed sequence(s)/gene(s) is to alter the accumulation of a specific endogenous messenger RNA (mRNA) or protein.

39. In addition, information should be provided to:

A. demonstrate whether the arrangement of the genetic material used for insertion has been conserved or whether significant rearrangement have occurred upon integration;
B. demonstrate whether deliberate modifications made to the amino acid sequence of the expressed protein result in changes in its post-translational modification or affected sites critical for its structure or function;
C. demonstrate whether the intended effect of the modification has been achieved and that all expressed traits are stable and are expressed as expected. It may be necessary to examine the inheritance of the DNA insert itself or the expression of the corresponding RNA if the phenotypic characteristics cannot be measured directly;
D. demonstrate whether the newly expressed trait(s) are expressed as expected in the appropriate tissues in a manner and at levels that are consistent with the associated regulatory sequences driving the expression of the corresponding gene;
E. indicate whether there is any evidence to suggest that one gene (or several genes) in the recombinant-DNA animal has been affected by the transformation process; and
F. confirm the identity and expression pattern of any new fusion proteins.

Safety assessment of the recombinant-DNA animal ultimately used as food or for food production

Health status of the recombinant-DNA animal

40. In contrast to the situation with plants, animals that have a history of safe use as sources of food generally do not contain genes encoding for toxic substances. Because of this, the health of a conventional animal has traditionally been used as a useful indicator of the safety of derived foods. The practice of only allowing animals with known and acceptable health status to enter the human food supply has been and continues to be an essential step in ensuring safe food.

41. An evaluation of the health of the animal is one of the essential steps in ensuring safety of food derived from recombinant-DNA animals. In undertaking this evaluation,

it is important to compare the health status of the recombinant-DNA animal with the health status of the appropriate conventional counterpart, taking into account developmental stage.

42. The evaluation should include the following:
A. general health and performance indicators, including behaviour, growth and development, general anatomy, and reproductive function, if appropriate;
B. physiological measures, including clinical and analytical parameters;
C. other species-specific considerations, where appropriate.

Expressed substances (non-nucleic acid substances)
Assessment of possible toxicity or bioactivity

43. *In vitro* nucleic acid techniques enable the introduction of DNA that can result in the synthesis of new substances in recombinant-DNA animals. The new substances can be conventional components of animal-derived foods, such as proteins, fats, carbohydrates and vitamins, that are novel in the context of that recombinant-DNA animal. New substances might also include new metabolites resulting from the activity of enzymes generated by the expression of introduced DNA.

44. It is recognized that the evaluation of the health status of the recombinant-DNA animals may give information about possible toxicity and bioactivity of the expressed substances. However, it is still generally expected that the safety assessment will include evaluation of these substances.

45. The safety assessment should take into account the chemical nature and function of the newly expressed substance and identify the concentration of the substance in the edible tissues and other derived food products of the recombinant-DNA animal, including variations and mean values. Current dietary exposure and possible effects on population subgroups should also be considered.

46. Information should be provided to ensure that genes coding for known toxins or antinutrients present in donor organisms, if applicable, are not transferred to recombinant-DNA animals that do not normally express those toxic or antinutritious characteristics. This assurance is particularly important in cases where food derived from the recombinant-DNA animal is processed differently from the donor organism, as conventional food processing techniques associated with the donor organisms may deactivate, degrade or eliminate antinutrients or toxicants.

47. For the reasons described in Section 3, conventional toxicology studies may not be considered necessary where the substance or a closely related substance has, taking into account its function and exposure, been consumed safely in food. In other cases, the use of appropriate conventional toxicology or other studies on the new substances may be necessary.

48. In the case of proteins, the assessment of potential toxicity should focus on amino acid sequence similarity between the protein and known protein toxins as well as stability

to heat or processing and to degradation in appropriate representative gastric and intestinal model systems. Appropriate oral toxicity studies[9] may need to be carried out in cases where the protein present in the food is not similar to proteins that have previously been consumed safely in food, taking into account its biological function in the animal where known.

49. Potential toxicity of non-protein substances that have not been safely consumed in food should be assessed on a case-by-case basis depending on the identity and biological function in the animal of the substance and dietary exposure. The type of studies to be performed may include studies on metabolism, toxicokinetics, subchronic toxicity, chronic toxicity/carcinogenicity, reproduction and development toxicity according to the traditional toxicological approach.

50. In the case of newly expressed bioactive substances, recombinant-DNA animals should be evaluated for potential effects of those substances as part of the overall animal health evaluation. It is possible that such substances may be active in humans. Therefore, consideration should be given to potential dietary exposure to the substance, whether the substance is likely to be bioactive following consumption and, if so, its potential to exert effects in humans.

51. Assessment of potential toxicity may require the isolation of the new substance from the recombinant-DNA animal, or the synthesis or production of the substance from an alternative source, in which case, the material should be shown to be biochemically, structurally and functionally equivalent to that produced in the recombinant-DNA animal.

Assessment of possible allergenicity (proteins)

52. When the protein(s) resulting from the inserted gene is present in the food, it should be assessed for potential allergenicity in all cases. An integrated, stepwise, case-by-case approach used in the assessment of the potential allergenicity of the newly expressed protein(s) should rely upon various criteria used in combination (as no single criterion is sufficiently predictive on either allergenicity or non-allergenicity). As noted in paragraph 21, the data should be obtained using sound scientific methods. A detailed presentation of issues to be considered can be found in the Annex to this document.[10]

53. The transfer of genes from commonly allergenic foods should be avoided unless it is documented that the transferred gene does not code for an allergen.

[9] Guidelines for oral toxicity studies have been developed in international fora, for example, the *OECD Guidelines for the Testing of Chemicals* issued by the Organisation for Economic Co-operation and Development.

[10] The FAO/WHO Expert Consultation 2001 report, which includes reference to several decision trees, was used in developing the Annex to this Guideline.

Compositional analysis of key components

54. Analyses of concentrations of key components[11] of the recombinant-DNA animal and, especially those typical of the food, should be compared with an equivalent analysis of a conventional counterpart grown and bred under the same husbandry conditions. Depending on the species (and the nature of the modification), it may be necessary to make comparisons between products from recombinant-DNA animals and appropriate conventional counterparts raised under more than one set of typical husbandry conditions. The statistical significance of any observed differences should be assessed in the context of the range of natural variations for that parameter to determine its biological significance. However, it should be acknowledged that, particularly in the case of certain animal species, the available number of samples may be limited and there is likely to be large variation between animals, even those bred and raised under the same husbandry conditions. The comparator(s) used in this assessment should ideally be matched in housing and husbandry conditions, breed, age, sex, parity, lactation, or laying cycle (where appropriate). In practice, this may not be feasible at all times, in which case, conventional counterparts as close as possible should be chosen. The purpose of this comparison, in conjunction with an exposure assessment as necessary, is to establish that substances that are nutritionally important or that can affect the safety of the food have not been altered in a manner that would have an adverse impact on human health.

Food storage and processing

55. The potential effects of food processing, including home preparation, on foods derived from recombinant-DNA animals should also be considered. For example, alterations could occur in the heat stability of a toxicant or the bioavailability of an important nutrient after processing. Therefore, information should be provided, describing the processing conditions used in the production of a food ingredient from the animal.

56. If the modification is intended to change storage or shelf-life, the impact of the modification on food safety and/or nutritional quality should be evaluated.

Intended nutritional modification

57. The assessment of possible compositional changes to key nutrients, which should be conducted for all recombinant-DNA animals, has already been addressed under "compositional analyses of key components". However, foods derived from recombinant-DNA animals that have undergone modification to alter nutritional quality or functionality intentionally should be subjected to additional nutritional assessment 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.

[11] Key nutrients are those components in a particular food that may have a substantial impact in the overall diet. They may be major constituents (fats, proteins, carbohydrates as nutrients or enzyme inhibitors as antinutrients) or minor compounds (minerals, vitamins). Key toxicants are those toxicologically significant compounds known to be inherently present in the organism, such as those compounds whose toxic potency and level may be significant to health and allergens. In animals, the presence of toxicants would be rare, whereas the presence of allergens would be common in some species.

58. Information about the known patterns of use and consumption of a food and its derivatives should be used to estimate the likely intake of the food derived from the recombinant-DNA animal. The expected intake of the food should be used to assess the nutritional implications of the altered nutrient profile both at customary and maximal levels of consumption. Basing the estimate on the highest likely consumption provides assurance that the potential for any undesirable nutritional effects will be detected. Attention should be paid to the particular physiological characteristics and metabolic requirements of specific population groups such as infants, children, pregnant and lactating women, the elderly and those with chronic diseases or compromised immune systems. Based on the analysis of nutritional impacts and the dietary needs of specific population subgroups, additional nutritional assessments may be necessary. It is also important to ascertain to what extent the modified nutrient is bioavailable and remains stable with time, processing and storage.

59. The use of animal breeding, including *in vitro* nucleic acid techniques, to change nutrient levels in animal-derived foods can result in broad changes to the nutrient profile in two ways. The intended modification in animal constituents could change the overall nutrient profile of the animal product, and this change could affect the nutritional status of individuals consuming the food. Unexpected alterations in nutrients could have the same effect. Although the recombinant-DNA animal components may be individually assessed as safe, the impact of the change on the overall nutrient profile should be determined.

60. When the modification results in a food product with a composition that is significantly different from its conventional counterpart, it may be appropriate to use additional conventional foods or food components (i.e. foods or food components whose nutritional composition is closer to that of the food derived from the recombinant-DNA animal) as appropriate comparators to assess the nutritional impact of the food.

61. Because of geographical and cultural variation in food consumption patterns, nutritional changes to a specific food may have a greater impact in some geographical areas or in some cultural populations than in others. Some animal-derived foods serve as the major source of a particular nutrient in some populations. The nutrient and the populations affected should be identified.

62. Some foods may require additional testing. For example, animal feeding studies may be warranted for foods derived from recombinant-DNA animals if changes in the bioavailability of nutrients are expected or if the composition is not comparable with conventional foods. In addition, foods designed for health benefits may require specific nutritional, toxicological or other appropriate studies. If the characterization of the food indicates that the available data are insufficient for a thorough safety assessment, properly designed animal studies could be requested on the whole foods.

SECTION 5 – OTHER CONSIDERATIONS

Potential altered accumulation or distribution of substances or micro-organisms significant to human health

63. Some recombinant-DNA animals may exhibit traits that may result in the potential for altered accumulation or distribution of xenobiotics (e.g. veterinary drug residues, metals), which may affect food safety. Similarly, the potential for altered colonization by and shedding of human pathogens or new symbiosis with toxin-producing organisms in the recombinant-DNA animal could have an effect on food safety. The safety assessment should take the potential for these alterations into account, and where such alterations are identified, consideration should be given to the potential impacts on human health using conventional procedures for establishing safety.

Use of antibiotic resistance marker genes

64. Alternative transformation technologies that do not result in antibiotic resistance marker genes in foods should be used in the future development of recombinant-DNA animals, where such technologies are available and demonstrated to be safe.

65. Gene transfer from animals and their food products to gut micro-organisms or human cells is considered a rare possibility because of the many complex and unlikely events that would need to occur consecutively. Nevertheless, the possibility of such events cannot be completely discounted.[12]

66. In assessing safety of foods containing antibiotic resistance marker genes, the following factors should be considered:

A. the clinical and veterinary use and importance of the antibiotic in question; (Certain antibiotics are the only drug available to treat some clinical conditions [e.g. vancomycin for use in treating certain staphylococcal infections]. Marker genes encoding resistance to such antibiotics should not be used in recombinant-DNA animals.)

B. whether the presence in food of the enzyme or protein encoded by the antibiotic resistance marker gene would compromise the therapeutic efficacy of orally administered antibiotic; and (This assessment should provide an estimate of the amount of orally ingested antibiotic that could be degraded by the presence of the enzyme in food, taking into account factors such as dosage of the antibiotic, amount of enzyme likely to remain in food following exposure to digestive conditions, including neutral or alkaline stomach conditions and the need for enzyme cofactors, e.g. adenosine triphosphate [ATP] for enzyme activity and estimated concentration of such factors in food.)

C. safety of the gene product, as would be the case for any other expressed gene product.

[12] In cases where there are high levels of naturally occurring bacteria that are resistant to the antibiotic, the likelihood of such bacteria transferring this resistance to other bacteria will be orders of magnitude higher than the likelihood of transfer between ingested foods and bacteria.

67. If evaluation of the data and information suggests that the presence of the antibiotic resistance marker gene or gene product presents risks to human health, the marker gene or gene product should not be present in foods. Antibiotic resistance genes used in food production that encode resistance to clinically used antibiotics should not be present in foods.

Review of safety assessments

68. The goal of the safety assessment is a conclusion as to whether the new food is as safe as the conventional counterpart taking into account dietary impact of any changes in nutritional content or value. Nevertheless, the safety assessment should be reviewed in the light of new scientific information that calls into question the conclusions of the original safety assessment.

ANNEX

ASSESSMENT OF POSSIBLE ALLERGENICITY

SECTION 1 – INTRODUCTION

1. All newly expressed proteins[13] in recombinant-DNA animals that could be present in the final food should be assessed for their potential to cause allergic reactions. This should include consideration of whether a newly expressed protein is one to which certain individuals may already be sensitive as well as whether a protein new to the food supply is likely to induce allergic reactions in some individuals.

2. At present, there is no definitive test that can be relied upon to predict allergic response in humans to a newly expressed protein. Therefore, it is recommended that an integrated, stepwise, case-by-case approach, as described below, be used in the assessment of possible allergenicity of newly expressed proteins. This approach takes into account the evidence derived from several types of information and data as no single criterion is sufficiently predictive.

3. The end-point of the assessment is a conclusion as to the likelihood of the protein being a food allergen.

SECTION 2 – ASSESSMENT STRATEGY

4. The initial steps in assessing possible allergenicity of any newly expressed proteins are the determination of: the source of the introduced protein; any significant similarity between the amino acid sequence of the protein and that of known allergens; and its structural properties, including, but not limited to, its susceptibility to enzymatic degradation, heat stability and/or acid and enzymatic treatment.

5. As there is no single test that can predict the likely human immunoglobulin E (IgE) response to oral exposure, the first step to characterize newly expressed proteins should be the comparison of the amino acid sequence and certain physicochemical characteristics of the newly expressed protein with those of established allergens in a weight of evidence approach. This will require the isolation of any newly expressed proteins from the recombinant-DNA animal, or the synthesis or production of the substance from an alternative source, in which case, the material should be shown to be structurally, functionally and biochemically equivalent to that produced in the recombinant-DNA animal. Particular attention should be given to the choice of the expression host, as post-translational modifications allowed by different hosts (i.e. eukaryotic vs prokaryotic systems) may have an impact on the allergenic potential of the protein.

[13] This assessment strategy is not applicable to the evaluation of foods where gene products are down regulated for hypoallergenic purposes.

6. It is important to establish whether the source is known to cause allergic reactions. Genes derived from known allergenic sources should be assumed to encode an allergen unless scientific evidence demonstrates otherwise.

SECTION 3 – INITIAL ASSESSMENT

Section 3.1 – Source of the protein

7. As part of the data supporting the safety of foods derived from recombinant-DNA animals, information should describe any reports of allergenicity associated with the donor organism. Allergenic sources of genes would be defined as those organisms for which reasonable evidence of IgE mediated oral, respiratory or contact allergy is available. Knowledge of the source of the introduced protein allows the identification of tools and relevant data to be considered in the allergenicity assessment. These include: the availability of sera for screening purposes; documented type, severity and frequency of allergic reactions; structural characteristics and amino acid sequence; physicochemical and immunological properties (when available) of known allergenic proteins from that source.

Section 3.2 – Amino acid sequence homology

8. The purpose of a sequence homology comparison is to assess the extent to which a newly expressed protein is similar in structure to a known allergen. This information may suggest whether that protein has an allergenic potential. Sequence homology searches comparing the structure of all newly expressed proteins with all known allergens should be done. Searches should be conducted using various algorithms such as FASTA or BLASTP to predict overall structural similarities. Strategies such as stepwise contiguous identical amino acid segment searches may also be performed for identifying sequences that may represent linear epitopes. The size of the contiguous amino acid search should be based on a scientifically justified rationale in order to minimize the potential for false negative or false positive results.[14] Validated search and evaluation procedures should be used in order to produce biologically meaningful results.

9. IgE cross-reactivity between the newly expressed protein and a known allergen should be considered a possibility when there is more than 35 percent identity in a segment of 80 or more amino acids (FAO/WHO 2001) or other scientifically justified criteria. All the information resulting from the sequence homology comparison between the newly expressed protein and known allergens should be reported to allow a case-by-case scientifically based evaluation.

10. Sequence homology searches have certain limitations. In particular, comparisons are limited to the sequences of known allergens in publicly available databases and the scientific literature. There are also limitations in the ability of such comparisons to

[14] It is recognized that the 2001 FAO/WHO consultation suggested moving from 8 to 6 identical amino acid segments in searches. The smaller the peptide sequence used in the stepwise comparison, the greater the likelihood of identifying false positives; inversely, the larger the peptide sequence used, the greater the likelihood of false negatives, thereby reducing the utility of the comparison.

detect non-contiguous epitopes capable of binding themselves specifically with IgE antibodies.

11. A negative sequence homology result indicates that a newly expressed protein is not a known allergen and is unlikely to be cross-reactive to known allergens. A result indicating absence of significant sequence homology should be considered along with the other data outlined under this strategy in assessing the allergenic potential of newly expressed proteins. Further studies should be conducted as appropriate (see also Sections 4 and 5). A positive sequence homology result indicates that the newly expressed protein is likely to be allergenic. If the product is to be considered further, it should be assessed using serum from individuals sensitized to the identified allergenic source.

Section 3.3 – Pepsin resistance

12. Resistance to pepsin digestion has been observed in several food allergens; thus, a correlation exists between resistance to digestion by pepsin and allergenic potential.[15] Therefore, the resistance of protein to degradation in the presence of pepsin under appropriate conditions indicates that further analysis should be conducted to determine the likelihood of the newly expressed protein being allergenic. The establishment of a consistent and well-validated pepsin degradation protocol may enhance utility of this method. However, it should be taken into account that a lack of resistance to pepsin does not exclude that the newly expressed protein can be a relevant allergen.

13. Although the pepsin resistance protocol is strongly recommended, it is recognized that other enzyme susceptibility protocols exist. Alternative protocols may be used where adequate justification is provided.[16]

SECTION 4 – SPECIFIC SERUM SCREENING

14. For those proteins that originate from a source known to be allergenic, or have sequence homology with a known allergen, testing in immunological assays should be performed where sera are available. Sera from individuals with a clinically validated allergy to the source of the protein can be used to test the specific binding to IgE class antibodies of the protein in *in vitro* assays. A critical issue for testing will be the availability of human sera from sufficient number of individuals.[17] In addition, the quality of the sera and the assay procedure need to be standardized to produce a valid test result. For proteins from sources not known to be allergenic and which do not

[15] The method outlined in *The United States Pharmacopoeia* (1995) was used in the establishment of the correlation (Astwood ***et al***., 1996).

[16] Report of the Joint FAO/WHO Expert Consultation on the allergenicity of foods derived from biotechnology (2001): *Evaluation of allergenicity of genetically modified foods*, Section 6.4 Pepsin resistance.

[17] According to the report of the Joint FAO/WHO Expert Consultation on allergenicity of foods derived from biotechnology (22–25 January 2001, Rome) a minimum of eight relevant sera is required in order to achieve a 99 percent certainty that the new protein is not an allergen in the case of a major allergen. Similarly, a minimum of 24 relevant sera is required to achieve the same level of certainty in the case of a minor allergen. It is recognized that these quantities of sera may not be available for testing purposes.

exhibit sequence homology to a known allergen, targeted serum screening may be considered where such tests are available as described in paragraph 17.

15. In the case of a newly expressed protein derived from a known allergenic source, a negative result in *in vitro* immunoassays may not be considered sufficient but should prompt additional testing, such as the possible use of skin test and *ex vivo* protocols.[18] A positive result in such tests would indicate a potential allergen.

SECTION 5 – OTHER CONSIDERATIONS

16. The absolute exposure to the newly expressed protein and the effects of relevant food processing will contribute towards an overall conclusion about the potential for human health risk. In this regard, the nature of the food product intended for consumption should be taken into consideration in determining the types of processing that would be applied and its effects on the presence of the protein in the final food product.

17. As scientific knowledge and technology evolve, other methods and tools may be considered in assessing the allergenicity potential of newly expressed proteins as part of the assessment strategy. These methods should be scientifically sound and may include: targeted serum screening (i.e. the assessment of binding to IgE in sera of individuals with clinically validated allergic responses to broadly-related categories of foods); the development of international serum banks; use of animal models; and examination of newly expressed proteins for T-cell epitopes and structural motifs associated with allergens.

[18] *Ex vivo* procedure is described as the testing for allergenicity using cells or tissue culture from allergic human subjects (report of Joint FAO/WHO Expert Consultation on allergenicity of foods derived from biotechnology).

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Foods derived from modern biotechnology

The texts in this publication represent the outcome of the work of the Codex Alimentarius Commission on principles and guidelines for food safety assessment of foods derived from modern biotechnology. They give guidance on how to assess the safety of such foods and thus protect the health of consumers. This second edition includes texts adopted by the Codex Alimentarius Commission up to 2008.

The Codex Alimentarius Commission is an intergovernmental body with more than 180 members, within the framework of the Joint FAO/WHO Food Standards Programme established by the Food and Agriculture Organization (FAO) of the United Nations and the World Health Organization (WHO). The main result of the Commission's work is the *Codex Alimentarius,* a collection of internationally adopted food standards, guidelines, codes of practice and other recommendations, with the objective of protecting the health of consumers and ensuring fair practices in the food trade.

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