

<p>GUIDELINE FOR THE CONDUCT OF FOOD SAFETY ASSESSMENT OF FOODS DERIVED FROM RECOMBINANT-DNA PLANTS CAC/GL 45-2003 Adopted in 2003, Annexes II and III adopted in 2008.</p>	<p>GUIDELINE FOR THE CONDUCT OF FOOD SAFETY ASSESSMENT OF FOODS DERIVED FROM RECOMBINANT-DNA ANIMALS CAC/GL 68-2008</p>
<p>SECTION 1 - SCOPE</p> <p>1. This Guideline supports the Principles for the Risk Analysis of Foods Derived from Modern Biotechnology. 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.</p> <p>2. This document does not address animal feed or animals fed with the feed. This document also does not address environmental risks.</p>	<p>SECTION 1 - SCOPE</p> <p>1. This Guideline supports the Principles for the Risk Analysis of Foods Derived from Modern Biotechnology. 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 traits¹.</p> <p>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-DNA methods in developing animals for food use might affect those issues, this Guideline addresses only food safety and nutritional issues. It therefore does not address:</p> <ul style="list-style-type: none"> • animal welfare; • ethical, moral and socio-economical 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 microorganisms.

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 likely 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-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. 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 before it is considered for commercial distribution.

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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 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. 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 before it is 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.

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.

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.

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 techniques³.

8. A diverse range of animals are 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

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 food¹.

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, soya, 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 endpoints is a major element in the risk assessment of many compounds such as pesticides. In most

framework presented in this Guideline.

SECTION 2 - DEFINITIONS

9. The definitions below apply to this Guideline:

“Recombinant-DNA Animal” — 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” — 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 animals⁴.

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 endpoints is a major element in the risk assessment of many compounds, such as pesticides. In most

cases, however, the substance to be tested is well characterised, of known purity, of no particular nutritional value, and, human exposure to it is generally low. It is therefore 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.

11. Animal studies cannot readily be applied to testing the risks associated with whole foods, which are complex mixtures of compounds, often characterised by a wide variation in composition and nutritional value. Due 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 which 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. Due to the difficulties of applying traditional toxicological testing and risk assessment procedures to whole foods, a more focused approach is required for the

cases, however, the substance to be tested is well characterized, of known purity, of no particular nutritional value, and human exposure to it is generally low. It is therefore 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. Due 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 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. Due to the difficulties of applying traditional toxicological testing and risk assessment procedures to whole foods, and based on the experience of assessing 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 which 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 which 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 counterpart². 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 also occur in conventional breeding. Unintended effects may

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, which 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, which 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 counterpart⁵. 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

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 due to 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. Due to the expanding information on plant genome 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

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.

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 due to *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

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 are 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, 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);

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 are 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, 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 modification⁶ and its use as food or for food production;
- C) Description of the donor organism or other source(s) of the introduced recombinant-DNA;

<p>F) Safety assessment:</p> <ul style="list-style-type: none"> a) expressed substances (non-nucleic acid substances); b) compositional analyses of key components; c) evaluation of metabolites ; d) food processing; e) nutritional modification; and <p>G) Other considerations.</p>	<p>D) Description of the genetic modification(s) including the construct(s) used to introduce the recombinant-DNA;</p> <p>E) Description of the methods used to produce the initial recombinant-DNA animal⁷ and the processes to produce the recombinant-DNA animal ultimately used as food or for food production;</p> <p>F) Characterization of the genetic modification(s) in the recombinant-DNA animal ultimately used as food or for food production;</p> <p>G) Safety assessment:</p> <ul style="list-style-type: none"> 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; <p>H) Other considerations.</p>
<p>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.</p>	<p>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.</p>
<p>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.</p>	<p>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 at request. Data should be obtained using sound scientific methods and analysed using appropriate statistical techniques. Analytical methods should be documented.⁸</p>

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 endpoint 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.

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 endpoint 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 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 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 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 impact on human health ;
- C) information on the host plant’s genotype and phenotype 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 the plant’s 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 macro- or micro-nutrients it

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 impact on human health;
- C) information on the animal’s genotype and phenotype 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 its 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

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 anti-nutrients). 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, anti-nutrients and allergens; for microorganisms, 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).

what important macro- or micronutrients it contributes 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) Whether the recombinant-DNA was synthesized and it is not from a known natural source;
- B) If derived from another organism:
 - i. that organism's usual or common name;
 - 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 microorganisms, 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)	DESCRIPTION OF THE GENETIC MODIFICATION(S) INCLUDING THE CONSTRUCT(S) USED TO INTRODUCE THE RECOMBINANT-DNA
<p>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.</p>	<p>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.</p>
<p>28. The description of the transformation process should include:</p> <ul style="list-style-type: none"> 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. 	<p>29. The description of the process of introducing and incorporating (if appropriate) the recombinant-DNA into the recipient animal should include:</p> <ul style="list-style-type: none"> 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: <ul style="list-style-type: none"> - 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.
<p>29. Information should be provided on the DNA to be introduced, including:</p> <ul style="list-style-type: none"> 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; 	<p>30. Information should be provided on the DNA to be introduced, including:</p> <ul style="list-style-type: none"> 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,

<p>C) the location and orientation of the sequence in the final vector/construct; and D) the function.</p>	<p>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.</p> <p>DESCRIPTION OF THE METHODS USED TO PRODUCE INITIAL RECOMBINANT-DNA ANIMAL AND THE PROCESSES TO PRODUCE THE RECOMBINANT DNA ANIMAL ULTIMATELY USED AS FOOD OR FOR FOOD PRODUCTION</p> <p>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.</p> <p>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).</p> <p>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.</p> <p>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</p>
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<p>CHARACTERIZATION OF THE GENETIC MODIFICATION(S)</p> <p>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.</p> <p>31. Information should be provided on the DNA insertions into the plant genome; this should include:</p> <ul style="list-style-type: none"> A) the characterization and description of the inserted genetic materials; B) the number of insertion sites; C) the organisation 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 	<p>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.</p> <p>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, its 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).</p> <p>CHARACTERIZATION OF THE GENETIC MODIFICATION(S) IN THE RECOMBINANT-DNA ANIMAL ULTIMATELY USED AS FOOD OR FOR FOOD PRODUCTION</p> <p>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.</p> <p>37. Information should be provided on the DNA insertions into the animal genome; this should include:</p> <ul style="list-style-type: none"> 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
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<p>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</p> <p>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.</p> <p>32. Information should be provided on any expressed substances in the recombinant-DNA plant; this should include:</p> <ul style="list-style-type: none"> A) the gene product(s) (e.g. a protein or an untranslated RNA); B) the gene product(s)' function; 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 mRNA or protein. <p>33. In addition, information should be provided:</p> <ul style="list-style-type: none"> A) to demonstrate whether the arrangement of the genetic material used for insertion has been conserved or whether significant rearrangements have 	<p>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</p> <p>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.</p> <p>38. Information should be provided on any newly expressed substances in the recombinant-DNA animal; this should include:</p> <ul style="list-style-type: none"> A) the gene product(s) (e.g. a protein or an untranslated 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 gene product(s)' function; 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 mRNA or protein. <p>39. In addition, information should be provided to:</p> <ul style="list-style-type: none"> A) demonstrate whether the arrangement of the genetic material used for insertion has been conserved or whether significant rearrangement have
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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 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

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 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

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, vitamins which 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.

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 to 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 to 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, vitamins, which 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.

<p>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 sub-groups should also be considered.</p> <p>36. Information should be provided to ensure that genes coding for known toxins or anti-nutrients present in the donor organisms are not transferred to recombinant-DNA plants that do not normally express those toxic or anti-nutritious characteristics. This assurance is particularly important in cases where a recombinant-DNA plant is processed differently from a donor plant, since conventional food processing techniques associated with the donor organisms may deactivate, degrade or eliminate anti-nutrients or toxicants.</p> <p>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.</p>	<p>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.</p> <p>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 sub- groups should also be considered.</p> <p>46. Information should be provided to ensure that genes coding for known toxins or anti-nutrients present in donor organisms, if applicable, are not transferred to recombinant-DNA animals that do not normally express those toxic or anti-nutritious characteristics. This assurance is particularly important in cases where food derived from the recombinant-DNA animal is processed differently from the donor organism, since conventional food processing techniques associated with the donor organisms may deactivate, degrade or eliminate anti-nutrients or toxicants.</p> <p>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.</p>
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<p>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 anti-nutrients (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 studies³ 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.</p>	<p>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 studies⁹ 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.</p>
<p>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 type of studies to be performed may include studies on metabolism, toxicokinetics, sub-chronic toxicity, chronic toxicity/carcinogenicity, reproduction and development toxicity according to the traditional toxicological approach.</p>	<p>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, sub-chronic toxicity, chronic toxicity/carcinogenicity, reproduction and development toxicity according to the traditional toxicological approach.</p>
<p>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</p>	<p>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. Consideration should therefore 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.</p>
<p>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</p>	<p>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</p>

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 (since 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 document⁴.

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 components⁵ of the recombinant-DNA plant and, especially those typical of the food, should be compared with an equivalent

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 (since 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 document¹⁰.

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.

Compositional Analysis of Key Components

54. Analyses of concentrations of key components¹¹ of the recombinant-DNA animal and, especially those typical of the food, should be compared with an

<p>analysis 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 an 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.</p> <p>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 minimise 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.</p> <p>Evaluation of Metabolites</p> <p>46. Some recombinant-DNA plants may have been modified in a manner that could</p>	<p>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.</p>
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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. Information should therefore 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 intentionally alter nutritional quality or functionality should be subjected to additional nutritional

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. Information should therefore 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 intentionally alter nutritional quality or functionality 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. 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

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.

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

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 population 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. Also, 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.

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 population 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 to conventional foods. Also, 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) which may indirectly result in the potential for accumulation of pesticide residues, altered metabolites of such residues, toxic metabolites, contaminants, or other substances which 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 microorganisms 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 discounted⁶.

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

SECTION 5 – OTHER CONSIDERATIONS

POTENTIAL ALTERED ACCUMULATION OR DISTRIBUTION OF SUBSTANCES OR MICROORGANISMS 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 microorganisms 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 discounted¹².

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 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. ATP) for enzymatic 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.

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

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. 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.

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 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

68. The goal of the safety assessment is a conclusion as to whether the new food is

<p>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.</p>	<p>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.</p>
<p>footnote</p>	<p>footnote</p>
<p>1 It is recognized that for the foreseeable future, foods derived from modern biotechnology will not be used as conventional counterparts.</p> <p>2 The concept of substantial equivalence as described in the report of the 2000 joint FAO /WHO expert consultations (Document WHO/SDE/PHE/FOS/00.6, WHO, Geneva, 2000).</p> <p>3 Guidelines for oral toxicity studies have been developed in international fora, for example, the OECD Guidelines for the Testing of Chemicals.</p> <p>4 The FAO/WHO expert consultation 2001 report, which includes reference to several decision trees, was used in developing Annex 1 to these guidelines.</p> <p>5 Key nutrients or key anti-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 anti-nutrients) 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.</p>	<p>1 This Guideline was developed primarily for animals bearing heritable recombinant-DNA constructs.</p> <p>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.</p> <p>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.</p> <p>4 It is recognized that for the foreseeable future, foods derived from modern biotechnology will not be used as conventional counterparts.</p> <p>5 The concept of substantial equivalence as described in the report of the 2000 joint FAO/WHO expert consultation (Document WHO/SDE/PHE/FOS/00.6, WHO,</p>

6 In cases where there are high levels of naturally occurring bacteria which 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.

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.

6 Not to be confused with a surrogate dam.

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 Procedural Manual.

9 Guidelines for oral toxicity studies have been developed in international fora, for example, the OECD Guidelines for the Testing of Chemicals.

10 The FAO/WHO expert consultation 2001 report, which includes reference to several decision trees, was used in developing the Annex to these guidelines.

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 anti-nutrients) 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

	<p>of allergens would be common in some species.</p> <p>12 In cases where there are high levels of naturally occurring bacteria which 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.</p>
<p>ANNEX 1: ASSESSMENT OF POSSIBLE ALLERGENICITY</p>	<p>ANNEX: ASSESSMENT OF POSSIBLE ALLERGENICITY</p>
<p>SECTION 1 – INTRODUCTION</p> <p>1. All newly expressed proteins⁷ 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.</p> <p>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 since no single criterion is sufficiently predictive.</p> <p>3. The endpoint of the assessment is a conclusion as to the likelihood of the protein being a food allergen.</p>	<p>SECTION 1 – INTRODUCTION</p> <p>1. All newly expressed proteins¹³ 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.</p> <p>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 since no single criterion is sufficiently predictive.</p> <p>3. The endpoint of the assessment is a conclusion as to the likelihood of the protein being a food allergen.</p>

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 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, since post-translational modifications allowed by different hosts (i.e.: eukaryotic vs. prokaryotic systems) may have an impact on the allergenic potential of the protein.

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.

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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.

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9. IgE cross-reactivity between the newly expressed protein and a known allergen should be considered a possibility when there is more than 35% 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 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

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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 provided¹⁰.

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 individuals¹¹ 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 exhibit sequence homology to a known allergen, targeted serum screening may be considered where such tests are available as described in paragraph ¹⁷.

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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 toward 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 which would be applied and its effects on the presence of the protein in the final food product.

17. As scientific knowledge and technology evolves, 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.

footnote

⁷ This assessment strategy is not applicable for assessing whether newly expressed proteins are capable of inducing glutensensitive 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

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footnote

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

¹⁴ It is recognized that the 2001 FAO/WHO consultation suggested moving from 8

<p>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.</p> <p>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.</p> <p>9 The method outlined in the U.S. Pharmacopoeia (1995) was used in the establishment of the correlation (Astwood et al. 1996).</p> <p>10 Report of Joint FAO/WHO Expert Consultation on Allergenicity of Foods Derived from Biotechnology (2001): Section "6.4 Pepsin Resistance".</p> <p>11 According to the Joint Report of the FAO/WHO Expert Consultation on Allergenicity of Foods Derived from Biotechnology (22-25 January 2001, Rome, Italy) a minimum of 8 relevant sera is required to achieve a 99% 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.</p> <p>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</p>	<p>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.</p> <p>15 The method outlined in the U.S. Pharmacopoeia (1995) was used in the establishment of the correlation (Astwood et al. 1996).</p> <p>16 Report of the Joint FAO/WHO Expert Consultation on Allergenicity of Foods Derived from Biotechnology (2001): Section "6.4 Pepsin Resistance".</p> <p>17 According to the Report of the Joint FAO/WHO Expert Consultation on Allergenicity of Foods Derived from Biotechnology (22-25 January 2001, Rome, Italy) a minimum of 8 relevant sera is required to achieve a 99% 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.</p> <p>18 Ex vivo procedure is described as the testing for allergenicity using cells or tissue culture from allergic human subjects (Report of the Joint FAO/WHO Expert Consultation on Allergenicity of Foods derived from Biotechnology).</p>
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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 Codex Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants (CAC/GL 45-2003) (Codex 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.

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), or 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 - means any substance normally consumed as a constituent of food:

(a) which provides energy; or

(b) which 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.

SECTION 3 – FOOD SAFETY ASSESSMENT

5. The Codex 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 which 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 Codex Plant Guideline 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/sub populations, while other populations/sub populations may be at risk from the same food.

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. Since 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 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 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 recombinant-DNA plants due 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 Codex 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 to 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.

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 Codex Plant Guideline.

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). When national or regional food consumption data is unavailable, food availability data may provide a useful resource.

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, 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 characterise the distribution of exposures within the relevant population(s).

footnote

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)

15 Paragraphs 18-21 (Safety Framework) and 48-53 (Nutrition Modification)

16 Further guidance for susceptible and high-risk population groups is provided in paragraph 49 of the Codex Plant Guideline.

17 Codex Plant Guideline, paragraph 4

18 Codex Plant Guideline, paragraph 51

19 Where such guidance is not provided by Codex, information provided by the FAO/WHO may be preferably considered.

20 Additional applicable guidance on dietary exposure assessment of nutrients and related substances is provided in the Report of a Joint FAO/WHO Technical Workshop on Nutrient Risk Assessment. WHO Headquarters, Geneva, Switzerland, 2-6 May 2005.

21 Paragraphs 44 and 45

22 A Model for Establishing Upper Levels of Intake for Nutrients and Related Substances. Report of a 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 Codex Guideline for the conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants (CAC/GL 45-2003) (Codex 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.

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 oil seeds, 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 likely be the more common situation of low-level presence of recombinant-DNA plant material. Because any food serving of grains, beans or oil seeds 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.

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 Codex Plant Guideline. As a result, only certain elements of the Codex 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 Codex 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 countries' relevant import requirements,

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 Codex Plant Guideline apply as amended as follows. The applicable paragraphs are specifically indicated. Those paragraphs of the Codex Plant Guidelines that are not listed can be omitted from consideration.

DESCRIPTION OF THE RECOMBINANT-DNA PLANT

8. Paragraph 22 of the Codex Plant Guideline applies.

DESCRIPTION OF THE HOST PLANT AND ITS USE AS A FOOD

9. Paragraphs 23, 24 and 25 of the Codex 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 microorganisms,

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).

DESCRIPTION OF THE GENETIC MODIFICATION(S)

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

CHARACTERIZATION OF THE GENETIC MODIFICATION(S)

12. Paragraphs 30 and 31 of the Codex 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 gene product(s)' function;

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.

14. Paragraph 33 of the Codex 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.

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, since conventional food processing techniques associated with the donor organisms may deactivate, degrade or eliminate toxicants.

17. Paragraph 37 of the Codex 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 intestinal model systems. Appropriate oral toxicity studies²⁹ 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 Codex Plant Guideline apply.

Assessment of possible allergenicity (proteins)

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

Analyses of Key Toxicants and Allergens

21. Analyses of key toxicants³¹ 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.

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.

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 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).

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. Information should therefore 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.

POTENTIAL ACCUMULATION OF SUBSTANCES SIGNIFICANT TO
HUMAN HEALTH

25. Some recombinant-DNA plants may exhibit traits (e.g. herbicide tolerance) which may indirectly result in the potential for accumulation of pesticide residues, altered metabolites of such residues, toxic metabolites, contaminants, or other substances which 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.

USE OF ANTIBIOTIC RESISTANCE MARKER GENES

26. Paragraphs 55, 56, 57 and 58 of the Codex 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 Codex 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;
- 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 Codex 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; 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 Codex 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 Codex Plant Guideline by the authorizing Codex member should be made available.

footnote

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

25 The text of this paragraph was adapted from paragraph 26 of the Codex Plant Guideline.

26 The text of this paragraph was adapted from paragraph 32 of the Codex Plant Guideline.

27 The text of this paragraph was adapted from paragraph 35 of the Codex Plant Guideline.

28 The text of this paragraph was adapted from paragraph 36 of the Codex Plant Guideline.

29 Guidelines for oral toxicity studies have been developed in international fora, for example, the OECD Guidelines for the Testing of Chemicals.

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

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

34 The text of this paragraph was adapted from paragraph 46 of the Codex Plant Guideline.

35 The text of this paragraph was adapted from paragraph 47 of the Codex Plant Guideline.

36 The text of this paragraph was adapted from paragraph 54 of the Codex Plant Guideline.

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