

Global Low-Level Presence Initiative (GLI) Statement:

Principles, Criteria and Practical Approaches for Addressing Low-Level Presence (LLP) in International Food and Feed Trade of Plant Material

Recalling earlier statements to encourage development of practical approaches to address LLP globally and to work collaboratively to address the overarching problem of asynchronous authorizations, including the *International Statement on Low-Level Presence*, the *Communiqué on Approaches to Reduce Asynchronous Authorizations in Food and Feed Derived from Recombinant DNA (rDNA) Plants*, and the *Memorandum on Improving Access to Data and Information for Food and Feed Safety Assessment in Situations of Low-Level Presence (LLP) of Recombinant-DNA (rDNA) Plant Material*,

Acknowledging in accordance to the *International Statement on Low Level Presence*, for the purposes of this statement the definition of LLP for food as 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) in one or more countries, but 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; and recognizing that practical approaches need to cover both food and feed,

Noting that LLP is an issue of mounting importance, and that alongside the significant growth in global trade in agricultural products, a growing number of countries, including GLI members, are developing, commercializing, exporting, and importing rDNA plant products, and that predictable and reliable trade of food and feed helps contribute to innovation, development of new plant varieties, reduction in the environmental footprint of agriculture, and ultimately to an increase in global food security,

Recognizing that food and feed handling requires careful management to achieve specified quality; that it is unavoidable that bulk commodities may contain low levels of other materials and that occasional commingling in handling, along with biological factors, such as cross-pollination, can result in low levels of foreign materials, including rDNA plant products, in food and/or feed shipments,

Noting that disruptions to trade due to LLP, including those relating to risks of rejection of imports and regulatory non-compliance, can impact both exporting and importing countries in different ways, including reduced access to food and/or feed from imported sources, an inability to meet contractual requirements, and significant commercial losses to importing and exporting parties

Acknowledging that this proposal presents principles and approaches developed to give guidance on practical measures on LLP situations, while maintaining public trust and ensuring transparency;

Recognizing that because individual countries devise measures consistent with their domestic legal and regulatory requirements, each approach may not necessarily be applicable to every country,

In this context, underlining that this document is not binding and its aim is to suggest tools for addressing trade disruptions due to LLP,

We, the undersigned countries, support and encourage the acknowledgement of the following principles for the development of practical approaches to addressing LLP by governments and for bolstering continued industry stewardship efforts:

Guiding Principles for the Development of Practical Approaches to LLP

- Approaches to addressing LLP should recognize that, while food and feed are produced and handled to meet rigorous quality standards through reliance on management systems that help limit the potential for LLP, the scale of bulk food/feed handling and transport makes it virtually impossible to completely prevent LLP.
 - Approaches to addressing LLP should recognize that a zero tolerance policy for LLP may not be practicable under current food and/or feed handling systems and will become increasingly trade-disruptive as new rDNA plant products are approved and commercialized.
- Approaches to addressing LLP should strive to prevent LLP occurrences before they occur and reduce the likelihood and/or number of LLP occurrences.
 - Approaches to addressing LLP taken by governments would ideally work in concert with industry initiatives, including as those designed to prevent the occurrence of LLP.
 - Approaches to addressing LLP by industry should consider the potential for LLP occurrences as soon as a product is authorized in the country of origin but not yet in the country of import.
- Approaches to addressing LLP should recognize that the rDNA plant product of a potential or actual LLP occurrence has, by definition, received regulatory authorization for food and/or feed use in at least one country.
 - Approaches to addressing LLP should foster confidence and trust in safety decisions among countries.
 - Approaches to addressing LLP should take into account information supporting authorization of the rDNA plant product in other countries. Such information may be available at the Food and Agriculture Organization (FAO) of the United Nations Genetically Modified (GM) Foods Platform database or through product sponsors.

- Approaches to resolve actual LLP occurrences should encourage commercial entities to make available to importing countries relevant information about the safety of plant products that have received regulatory authorization in other countries.
- Approaches to addressing LLP should be science-based and practicable, facilitate predictable trade, minimize disruption to trade, and be consistent with relevant domestic law and regulations and international trade obligations.
- Approaches to addressing LLP should recognize that routine detection testing and monitoring of imports for LLP in food and/or feed is a complex and expensive process that may be prone to inaccuracies. Such detection testing and monitoring will become more challenging, both technically and practically, as well as costly, as more rDNA plant products are approved globally.

In addition to the above principles, we support and encourage the following essential elements of a given approach or approaches for addressing LLP, which include efforts by both governments and industry.

Essential Elements of Practical Approaches

- **Safety:** Before structuring an approach to mitigate the trade impact of LLP incidents, countries should consider how safety can be addressed. The food and feed safety of the rDNA plant product should be established, for example, by consideration of already completed safety assessments done either domestically or by other countries consistent with the Codex Alimentarius “Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants” or by conducting a science-based assessment taking into account, as applicable from the same guideline, “Annex 3: Food Safety Assessment in Situations of Low-Level Presence of Recombinant-DNA Plant Material in Food”. These safety assessments can inform risk management decisions by regulatory authorities and determination of appropriate measures for bringing an LLP occurrence into compliance.
- **Compliance:** Before an LLP occurrence, countries should put mechanisms in place to ensure that legal or compliance requirements to protect public health can be satisfied without unnecessarily disrupting trade.
- **Industry Stewardship:** Industry has an active and ongoing stewardship responsibility to ensure that only lawful products are traded in commerce. Practical approaches to addressing LLP developed by countries should fully take into account industry stewardship efforts and, where appropriate, build upon these efforts.

Based upon these principles and essential elements, we support consideration of the following possible approaches by governments and industry as practical means to address LLP. We recognize that these approaches require the establishment of various supporting mechanisms, and

that they could present challenges (e.g., technical, economic, or political) to governments and industry stakeholders.

Practical Approaches

Governments:

- Establish processes to take into account current information, including existing authorizations for full food and/or feed use of rDNA plant products.
 - Consider recognition of authorizations for food and/or feed use made by another country or countries, especially for plant products with a long track record of safety; such recognition could be applied as means of reducing asynchronous authorizations, addressing LLP, and improving credibility of and confidence in oversight decisions.
 - Consider using a list of rDNA plant products that have been reviewed for food and/or feed use by many regions and countries around the world and that are not likely to have adverse impacts on human or animal health as a resource to facilitate a recognition policy and/or inform decisions.
 - Cooperate with other governments, including regionally, to recognize authorizations.
 - Consider information from authorizations for food and/or feed use made by another country or countries.
 - Consider whether information from domestic authorizations for food and/or feed use of similar rDNA plant products is relevant for evaluating the risks posed by an LLP incident and, if so, apply such information, as applicable, to safety assessment of the unauthorized LLP material.
 - Increase communication among and between trading partners regarding new authorizations of rDNA plant products, so as to improve global information exchange.
 - Seek to develop and expand the use of technical dialogues with regulatory agencies from different countries to explore practical options for decision-making related to rDNA plant products.
 - Encourage year-round submission and review of applications for authorization of rDNA plants and plant products.
 - Promptly inform the importer or the importer's agent of an LLP occurrence and of any additional information required to allow the importing country to make a decision regarding an LLP occurrence;

- When available, provide to the exporting country a summary of any risk or safety assessment that the importing country has conducted in connection with the LLP occurrence;
- Work to synchronize authorizations of rDNA plant products over the long term and enhance confidence in authorizations made by other countries.
 - Where possible and appropriate, coordinate regulatory authorizations and develop mechanisms for alignment of regulatory processes.
- Pro-actively identify the potential for LLP situations to occur and provide mechanisms to proactively address food and/or feed safety in such situations.
- Pro-actively develop compliance measures for practical prevention and/or resolution of LLP occurrences. These could include establishment of trade-facilitative compliance thresholds under defined LLP circumstances that address the potential presence of trace amounts of foreign materials.

Governments and Industry:

- Establish mechanisms for early communication and active engagement with product developers, commercial interests, and other governments so that safety considerations can be established before an LLP occurrence or be confirmed expeditiously at the time of an occurrence.
- Ensure availability and accessibility to information on completed domestic food and/or feed safety assessments and authorizations, as national regulations and policies allow.

Industry:

- Conduct a trade assessment to identify key countries of production and import, prior to the commercialization of any new biotechnology product (crop by event).
- Work to synchronize the submission of applications of rDNA plant products over the long-term to reduce asynchronous authorizations.
- Meet applicable regulatory requirements in identified key countries for imports for each country of production prior to the commercialization of a new biotechnology product, unless determined otherwise in consultation with the value chain.
- Conduct early stage consultations (~ 3 years prior to projected commercialization) among the value chain to determine the production management method (e.g., identity preservation) to be used to minimize LLP occurrences after product commercialization.

This includes prompt and transparent communication on the product stewardship and supply chain management practices, and their implementation.

- Follow generally accepted best seed quality practices to prevent the presence of unauthorized products and minimize unintended incidental presence of products authorized in the country of production but not in the destination country.
- Make available prior to commercialization a reliable detection method or test for use by growers, processors and buyers that enables crop identity verification for intended use.

July 15, 2017

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

CAC/GL 45-2003

SECTION 1 - SCOPE

1. This Guideline supports the Principles for the Risk Analysis of Foods Derived from Modern Biotechnology. It addresses safety and nutritional aspects of foods consisting of, or derived from, plants that have a history of safe use as sources of food, and that have been modified by modern biotechnology to exhibit new or altered expression of traits.
2. This document does not address animal feed or animals fed with the feed. This document also does not address environmental risks.
3. The Codex principles of risk analysis, particularly those for risk assessment, are primarily intended to apply to discrete chemical entities such as food additives and pesticide residues, or a specific chemical or microbial contaminant that have identifiable hazards and risks; they are not intended to apply to whole foods as such. Indeed, few foods have been assessed scientifically in a manner that would fully characterise 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.
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.

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.

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

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 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 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 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.

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).

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 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);
- F) Safety assessment:
 - a) expressed substances (non-nucleic acid substances);
 - b) compositional analyses of key components;
 - c) evaluation of metabolites ;
 - d) food processing;
 - e) nutritional modification; and
- G) Other considerations.

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

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

21. The goal of each safety assessment is to provide assurance, in the light of the best available scientific knowledge, that the food does not cause harm when prepared, used and/or eaten according to its intended use. The expected 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.

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 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).

DESCRIPTION OF THE GENETIC MODIFICATION(S)

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

28. The description of the transformation process should include:

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

29. Information should be provided on the DNA to be introduced, including:

- A) the characterization of all the genetic components including marker genes, regulatory and other elements affecting the function of the DNA;
- B) the size and identity;
- C) the location and orientation of the sequence in the final vector/construct; and
- D) the function.

CHARACTERIZATION OF THE GENETIC MODIFICATION(S)

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

31. Information should be provided on the DNA insertions into the plant genome; this should include:
- A) the characterization and description of the inserted genetic materials;
 - B) the number of insertion sites;
 - C) the 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 a consequence of the inserted material, or, where more appropriate, other information such as analysis of transcripts or expression products to identify any new substances that may be present in the food; and
 - D) identification of any open reading frames within the inserted DNA or created by the insertions with contiguous plant genomic DNA including those that could result in fusion proteins.
32. Information should be provided on any expressed substances in the recombinant-DNA plant; this should include:
- A) the gene product(s) (e.g. a protein or an untranslated 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.
33. In addition, information should be provided:
- A) to demonstrate whether the arrangement of the genetic material used for insertion has been conserved or whether significant rearrangements have occurred upon integration;
 - B) to demonstrate whether deliberate modifications made to the amino acid sequence of the expressed protein result in changes in its post-translational modification or affect sites critical for its structure or function;
 - C) to demonstrate whether the intended effect of the modification has been achieved and that all expressed traits are expressed and inherited in a manner that is stable through several generations consistent with laws of inheritance. It may be necessary to examine the inheritance of the DNA insert itself or the expression of the corresponding RNA if the phenotypic characteristics cannot be measured directly;
 - D) to demonstrate whether the newly expressed trait(s) are expressed as expected in the appropriate tissues in a manner and at levels that are consistent with the associated regulatory sequences driving the expression of the corresponding gene;
 - E) to indicate whether there is any evidence to suggest that one or several genes in the host plant has been affected by the transformation process; and
 - F) to confirm the identity and expression pattern of any new fusion proteins.

SAFETY ASSESSMENT

Expressed Substances (non-nucleic acid substances)

Assessment of possible toxicity

34. In vitro nucleic acid techniques enable the introduction of DNA that can result in the synthesis of new substances in plants. The new substances can be conventional components of plant foods such as proteins, fats, carbohydrates, 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.

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.

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.

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

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

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.

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

Assessment of possible allergenicity (proteins)

41. When the protein(s) resulting from the inserted gene is present in the food, it should be assessed for potential allergenicity in all cases. An integrated, stepwise, case-by-case approach used in the assessment of the potential allergenicity of the newly-expressed protein(s) should rely upon various criteria used in combination (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 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.

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.

³ Guidelines for oral toxicity studies have been developed in international fora, for example, the OECD Guidelines for the Testing of Chemicals.

⁴ The FAO/WHO expert consultation 2001 report, which includes reference to several decision trees, was used in developing Annex 1 to these guidelines.

⁵ Key nutrients or key 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.

Evaluation of Metabolites

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

Food Processing

47. The potential effects of food processing, including home preparation, on foods derived from recombinant-DNA plants should also be considered. For example, alterations could occur in the heat stability of an endogenous toxicant or the bioavailability of an important nutrient after processing. 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 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 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.

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:

- 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 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.

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.

ANNEX 1: ASSESSMENT OF POSSIBLE ALLERGENICITY

SECTION 1 – INTRODUCTION

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

SECTION 2 - ASSESSMENT STRATEGY

4. The initial steps in assessing possible allergenicity of any newly expressed proteins are the determination of: the source of the introduced protein; any significant similarity between the amino acid sequence of the protein and that of known allergens; and its structural properties, including but not limited to, its susceptibility to enzymatic degradation, heat stability and/or, acid and enzymatic treatment.
5. As there is no single test that can predict the likely human 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.

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.

⁷ This assessment strategy is not applicable for assessing whether newly expressed proteins are capable of inducing gluten-sensitive or other enteropathies. The issue of enteropathies is already addressed in Assessment of possible allergenicity (proteins), paragraph 42 of the Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants. In addition, the strategy is not applicable to the evaluation of foods where gene products are down regulated for hypoallergenic purposes.

SECTION 3.2 – AMINO ACID SEQUENCE HOMOLOGY

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

9. IgE cross-reactivity between the newly expressed protein and a known allergen should be considered a possibility when there is more than 35% 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 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 individuals.¹¹ 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.

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.

⁹ The method outlined in the U.S. Pharmacopoeia (1995) was used in the establishment of the correlation (Astwood *et al.* 1996).

¹⁰ Report of Joint FAO/WHO Expert Consultation on Allergenicity of Foods Derived from Biotechnology (2001): Section "6.4 Pepsin Resistance".

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.

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.

¹² *Ex vivo* procedure is described as the testing for allergenicity using cells or tissue culture from allergic human subjects (Report of Joint FAO/WHO Expert Consultation on Allergenicity of Foods derived from Biotechnology).

ANNEX 2: FOOD SAFETY ASSESSMENT OF FOODS DERIVED FROM RECOMBINANT-DNA PLANTS MODIFIED FOR NUTRITIONAL OR HEALTH BENEFITS

SECTION 1 – INTRODUCTION

1. General guidance for the safety assessment of foods derived from recombinant-DNA plants is provided in the 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 measures¹³.
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*¹⁴ - 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 Guideline¹⁵ 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 food¹⁶.
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 counterpart¹⁷. 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 comparator¹⁸ 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.

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

8. Upper levels of intake for many nutrients that have been set out by some national, regional and international bodies¹⁹ 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 benefits²⁰.
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.²¹
16. Consumption patterns will vary from country to country depending on the importance of the food in the diet(s) of a given population(s). Therefore, it is recommended that consumption estimates are based on national or regional food consumption data when available, using existing guidance on estimation of exposure in a given population(s)²². When national or regional food consumption data is unavailable, food availability data may provide a useful resource²³.

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.

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).

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 circumstances²⁴.
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.

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.

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)²⁵.

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.²⁶

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.²⁷

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

17. Paragraph 37 of the Codex Plant Guideline applies.

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.

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.³²

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

Evaluation of Metabolites

23. Some recombinant-DNA plants may have been modified in a manner that could result in new or altered levels of various metabolites in the food. In certain cases of foods from recombinant-DNA plants (e.g., those that are commonly consumed whole and undiluted), consideration should be given to the potential for the accumulation of metabolites in the food that would adversely affect human health. Food safety assessment in situations of low level presence of recombinant-DNA material in foods from such plants requires investigation of residue and metabolite levels in the food. Where altered residue or metabolite levels are identified in foods, consideration should be given to the potential impacts on human health using conventional procedures for establishing the safety of such metabolites (e.g. procedures for assessing the human safety of chemicals in foods).³⁴

Food Processing

24. The potential effects of food processing, including home preparation, on foods derived from recombinant-DNA plants should also be considered. For example, alterations could occur in the heat stability of an endogenous toxicant. 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.³⁵

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.

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.³⁶

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 obtained³⁷; 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.

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.