Guideline for Food Safety Assessment of Genetically Modified Foods Derived from Recombinant-DNA Organisms

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(In case of any discrepancy between the Chinese text and the English translation thereof, the Chinese text shall govern.)

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Chapter One: General Provisions

I. Objective

This guideline explains the information required for food safety assessment of genetically modified foods. The original developers are required to submit necessary information to register their genetically modified organisms (hereafter as GMO) to the Ministry of Health and Welfare (MOHW) before introduce the genetically modified foods into market. This document also provides guideline for food manufacturer, food trader, and health authorities to evaluate food safety of the genetically modified foods.

II. Definitions

1. Gene Modification

Gene modification refers to the transferring of genetic materials or implant of live cells or organisms via genetic engineering, molecular biotechnology, or other related technologies to produce genetic recombination, exogenous genetic characteristics, or to suppress certain genes of the recipient. However, this does not include traditional breeding methods or techniques such as the merging, hybridization, mutation, *in-vitro* fertilization, somaclonal variation, and chromosome doubling of plants of the same species and protoplasts.

2. Genetically Modified Organism (GMO)

An organism with genetic material that has been altered by gene modification techniques.

3. Genetically Modified Foods (GMF)

Foods and food additives that are produced or manufactured from raw materials consisting of or containing genetically modified organisms.

4. Conventional Counterparts

A related variety/species, its components and/or products for which there is experience of establishing safety based on common use as food.

5. Host

An organism to which gene(s) or nuclear acid fragment(s) are inserted or transferred by gene modification techniques.

6. Vector

An agent that is used to deliver selected foreign gene (or nuclear acid fragment) into host for proliferation and expression.

7. Transferred or Introduced or Inserted Gene (or Nuclear Acid Fragment)

Any foreign gene (or nuclear acid fragment) that is transferred or introduced or inserted into host.

8. Gene Products

Any product derived from the transferred or introduced or inserted gene(s) (or nuclear acid fragment(s)).

9. Gene (or Nuclear Acid fragment) donor

An organism that provides gene(s) (or nuclear acid fragment(s)) for gene modification process.

10. Unintended Effects

By introducing a specific trait into an organism through the insertion of DNA sequences, additional traits may, in some cases, be acquired or existing traits be lost or modified.

11. Nutrients

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

12. Genetically modified Plants with Nutrition Improvement

Genetically modified plants exhibit a particular trait in portion(s) of the plant intended for food use; and the trait is a result of: (i) introduction of a new nutrient(s) or related substance(s), (ii) alteration of either the quantity or bioavailability of a nutrient(s) or related substance(s), (iii) removal or reduction of undesirable substance(s) (e.g. allergens or toxicants), or (iv) alteration of the interaction(s) of nutritional or health relevance of these substances.

13. Tolerable Upper Intake Levels (or Upper Levels or UL)

    The highest average daily nutrient intake level that is likely to pose no risk of adverse health effects to almost all (97-98%) individuals in the general population.

14. Bioavailability

    Bioavailability is the degree to which food nutrients are available for absorption and utilization in the body.

15. Acceptable Daily Intake (ADI)

    Acceptable daily intake is a measure of the amount of a specific substance in food or drinking water that can be ingested over a lifetime without an appreciable health risk. An ADI value is based on current research, with long-term studies on animals and observations of humans. First, a No Observable (Adverse) Effect Level, the amount of a substance that shows no toxic effects, is determined on the basis of animal studies intended to measure an effect at several doses. Usually the studies are performed with several doses including high doses. Then, the NOEL (or NOAEL) is scaled by a safety factor, conventionally 100, to account for the differences between test animals and humans (factor of 10) and possible differences in sensitivity between humans (another factor of 10). The ADI is usually given in mg per kg body weight per day.

16. Dietary Exposure Assessment

    Exposure assessment means the qualitative and/or quantitative evaluation of the likely intake of biological, chemical, and physical agents via food as well as exposures from other sources if relevant. Dietary exposure to (or intake of) food chemicals is estimated by combining food consumption data with food chemical concentration data. The general equation is:

$$\text{Dietary exposure (mg/kg body weight/day)} = \frac{\Sigma \left[\text{Concentration of chemical in food (mg/kg)} \times \text{Food consumption (kg/person/day)} \right]}{\text{Body weight (kg/person)}}$$

Chapter Two: Food Safety Assessment of Genetically Modified Foods

Food safety assessment of genetically modified foods is conducted in three phases as follows.

I. Phase I assessment is conducted according to the following information: Information of the GMO.

II. When the results of the phase I assessment indicate the presence of possible toxin(s) and/or allergen(s) in the genetically modified foods, it is necessary to carry out phase II assessment.

III. When phase I and phase II assessments of the genetically modified foods indicate that the available data are insufficient for a thorough safety assessment, properly designed animal studies are requested on the whole foods. Whole food is defined as the general edible parts of the genetically modified foods, of which the component has not been refined, processed, concentrated, or purified.

Chapter Three: Food Safety Assessment of Genetically Modified Plants

Food safety assessment of genetically modified plants is conducted in three phases as follows.

I. Phase I assessment is conducted according to the information of the genetically modified plants (Annex I and II)

II. When the results of the phase I assessment indicate the presence of possible toxin(s) and/or allergen(s) in the genetically modified plants, it is necessary to carry out phase II assessment. Phase II assessment is conducted according to the following information.

   A. Safety assessment of possible toxicity (Annex IV)

   B. Safety assessment of possible allergenicity (Annex V)

III. When phase I and phase II assessments of the genetically modified plants indicate that the available data are insufficient for a thorough safety assessment, properly designed animal studies are requested on the whole foods.

IV. Scope

   This Guideline 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. This document does not address genetically modified animal feed or animals fed with genetically modified feed, the beneficial or health claims of nutritionally enhanced genetically modified plants. This document also does not address environmental risks of genetically modified plants.

Chapter Four: Food Safety Assessment of Genetically Modified Microorganisms

Food safety assessment of genetically modified microorganisms is conducted in three phases as follows.

I. Phase I assessment is conducted according to the information of the genetically modified microorganisms (Annex III)

II. When the results of the phase I assessment indicate the presence of possible toxin(s) and/or allergen(s) in the genetically modified microorganisms, it is necessary to carry out phase II assessment. Phase II assessment is conducted according to the following information.

   A. Safety assessment of possible toxicity (Annex IV)

   B. Safety assessment of possible allergenicity (Annex V)

III. When phase I and phase II assessments of the genetically modified microorganisms indicate that the available data are insufficient for a thorough safety assessment, properly designed animal studies are requested on the whole foods.

IV. Scope

   This Guideline addresses safety and nutritional aspects of foods consisting of, or derived from, microorganisms that have a history of safe use as sources of food, which have been modified by modern biotechnology to exhibit new or altered traits. This document does not address safety of microorganisms used in agriculture (for plant protection, biofertilizers, in animal feed or food derived from animals fed the feed etc.), risks related to environmental releases of genetically modified microorganisms used in food production, safety of substances produced by microorganisms that are used as additives or processing aids, including enzymes for use in food production, specific purported health benefits or probiotic effects that may be attributed to the use of microorganisms in food, or issues relating to the safety of food production workers handling genetically modified microorganisms.

Annex I - Information Required for the Genetically Modified Plants

I. Application materials must include the following information:

A. Description of the genetically modified plant

A) Identity of the crop and the transformation event to be reviewed;

B) type and purpose of the genetic modification.

B. Description of the host plant and its use as food

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 plants genotype and phenotype relevant to its safety, including any known toxicity or allergenicity; and

D) history of safe use for consumption as food.

C. Description of the donor organism(s)

A) Its usual or common name, scientific name and taxonomic classification;

B) information about the natural history as concerns food safety

a. Information on naturally occurring toxins, anti-nutrients and allergens; for microorganisms, additional information on pathogenicity and the relationship to known pathogens; and

b. 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).

D. Description of the genetic modification(s)

A) 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.

B) Information should be provided on the DNA to be introduced, including:

a. the characterization of all the genetic components including marker genes,

b. regulatory and other elements affecting the 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.

E. Characterization of the genetic modification(s)

A) Information should be provided on the DNA insertions into the plant genome; this should include:

a. the characterization and description of the inserted genetic materials;

b. the number of insertion sites;

c. the organization of the inserted genetic material at each insertion site including copy number and sequence data of the inserted material and of the surrounding region, sufficient to identify any substances expressed as a consequence of the inserted material, or, where more appropriate, other information such as analysis of transcripts or expression products to identify any new substances that may be present in the food; and

d. identification of any open reading frames within the inserted DNA or created by the insertions with contiguous plant genomic DNA including those that could result in fusion proteins.

B) Information should be provided on any newly expressed substance(s) in the genetically modified plant.

This should include information listed from a. to e. If the

newly expressed substance(s) in the genetically modified plant are evaluated to be substantially equivalent to existing substances with known history of safe use as foods, no further assessment is required. Otherwise, assessment as listed in B) of F should be conducted.

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.

C) 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.

F. Safety assessment

A) Compositional analyses of key components

a. Key components are those components in a particular food that may have a substantial impact in the overall diet. They may be

a) major constituents (e.g. fats, proteins, carbohydrates);

b) minor compounds (e.g. minerals, vitamins);

c) anti-nutrients (e.g. enzyme inhibitors); and

d) key toxicants that 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.

b. Crop composition databases published by international organizations.

c. Analyses of concentrations of key components of the genetically modified plant, especially those typical of the food, should be compared with an equivalent analysis of a conventional counterpart grown and harvested under the same conditions. The comparison should meet the following requirements:

a) 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.

b) In some cases, a further comparison with the genetically modified plant grown under its expected agronomic conditions may need to be considered (e.g. application of an herbicide).

c) 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.

d. Experimental design

a) 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.

b) The number of trial sites should be sufficient to allow accurate assessment of compositional characteristics over this range.

c) Trials should be conducted over a sufficient number of generations to allow adequate exposure to the variety of conditions met in nature.

d) To minimise environmental effects, and to reduce any effect from naturally occurring genotypic variation within a crop variety, each trial site plots should be replicated and arranged randomly.

e) 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.

B) Initial assessment of possible toxicity and/or allergenicity of newly expressed substances in genetically modified plants.

a. The following information should be provided for initial assessment of possible toxicity of the newly expressed substances in genetically modified plants.

a) The concentration of the newly expressed substances in the edible parts of the genetically modified plant, including variations and mean values;

b) the chemical nature and function of the newly expressed substance; and

c) in the case of proteins, the assessment of potential toxicity should focus on

1. amino acid sequence similarity between the protein and known protein toxins and anti-nutrients (e.g. protease inhibitors, lectins);
2. stability to heat or processing; and
3. stability to degradation in appropriate representative gastric and intestinal model systems.

b. The following information should be provided for initial assessment of possible allergenicity of the newly expressed proteins in genetically modified plants.

a) The source of the introduced protein

1. Information should describe any reports of allergenicity associated with the donor organism.
2. When reasonable evidence of IgE mediated oral, respiratory or contact allergy of the donor organism is available, the following information may be provided.

1) Availability of sera for screening purposes;

2) documented type, severity and frequency of allergic reactions;

3) structural characteristics and amino acid sequence; and

4) physicochemical and immunological properties (when available) of known allergenic proteins from that source.

3. Genes derived from known allergenic sources should be assumed to encode an allergen unless scientific evidence demonstrates otherwise.
4. When genes are derived from non-allergenic sources, the following information should be provided.

1) Level and location of the newly expressed

protein; and

2) function of the newly expressed protein.

b) Amino acid sequence homology

1. 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 to predict overall structural similarities.

    1) Strategies such as stepwise contiguous identical amino acid segment searches may also be performed for identifying sequences that may represent linear epitopes.

    2) The newly expressed protein is considered significantly similar to a known allergen when more than 35% identity in a segment of 80 or more amino acids, contains linear epitope(s) or complies with other scientifically justified criteria, the sequence alignment result is positive.

2. 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, if available, from individuals allergic to the identified allergenic source. If there is no available serum, other scientifically sound methods and tools may be used to assess the allergenicity potential of the newly expressed protein(s).

c) Pepsin resistance

1. Resistance to pepsin digestion should be conducted with a consistent and well-validated pepsin degradation protocol.

2. Further analyses are required for a newly expressed protein with a pepsin digestion fragment larger than 3.5 kDa.

c. Alternative protocols may be used where adequate justification is provided. Initial assessment of possible toxicity and/or allergenicity of the newly expressed substances in genetically modified plants may require the isolation of the new substance from the genetically modified 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 genetically modified plant.

d. When the results of the initial assessment indicate that the newly expressed substance is possible toxin(s) and/or allergen(s), further assessment should be conducted according to Annex IV and/or V.

C) Evaluation of metabolites

Some genetically modified plants may have been modified in a manner that could result in new or altered levels of various metabolites in the food.

a. Consideration should be given to the potential for the accumulation of metabolites in the food that would adversely affect human health.

b. Safety assessment of such plants requires investigation of residue and metabolite levels in the food and assessment of any alterations in nutrient profile.

c. 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.

D) Nutritional modification

The following information should be provided for the assessment of genetically modified plants modified for nutritional or health benefits.

a. The likely intake of the genetically modified plant should be estimated. 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.

a) Upper levels of intake for many nutrients that have been set out by the Ministry of Health and Welfare

(MOHW) 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. 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.

b) 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.

b. Although the genetically modified plant components may be individually assessed as safe, the impact of the change on the overall nutrient profile should be determined.

c. 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 genetically modified plant) as appropriate comparators to assess the nutritional impact of the food.

d. It is also important to ascertain to what extent the modified nutrient is bioavailable and remains stable with time, processing and storage.

a) 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.

b) Bioavailability of the nutrient(s), related substance(s) or undesirable substance(s) in the food that were the subject of the modification in the genetically modified 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.

e. Animal feeding studies may be warranted for foods derived from genetically modified plants if changes in the bioavailability of nutrients are expected or if the composition is not comparable to conventional foods.

f. Dietary exposure to a nutrient(s) or related substance(s) should be evaluated in the context of the total diet and the assessment should be carried out based on the customary dietary consumption by the relevant population(s) of the corresponding food that is likely to be displaced. When evaluating the exposure, it is appropriate to consider information on whether the consumption of the modified food could lead to adverse nutritional effects as compared with consumption of the food that it is intended to replace.

   a) The concentration of the nutrient(s) or related substance(s) expressed in the portion of the plant intended for food use should be determined.

   b) Consumption estimates are based on food consumption data of Taiwan when available, using existing guidance on estimation of exposure in a given population(s). When the food consumption data are unavailable, the Food Balance Sheets issued by the Ministry of Agriculture (MOA) may provide a useful resource.

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

g. In consideration of geographical and cultural variation

in food consumption patterns, the nutrient and the populations affected should be identified for nutritional changes to a specific food.

h. 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.

i. 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.

E) Food processing

Information should be provided describing the processing conditions used in the production of a food ingredient from the genetically modified plant.

F) Antibiotic resistance marker genes

The following information should be provided for safety assessment of the antibiotic resistance marker genes.

a. Describe the clinical and veterinary use and importance of the antibiotic in question;

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;

c. safety of the gene product; and

d. documentation, if any, regarding the approval and consumption of the antibiotic resistance marker genes in other countries. Any other information available to demonstrate the safety of the antibiotic resistance marker genes.

G) Other considerations

a. Some genetically modified 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.

b. The safety assessment should be reviewed in the light of new scientific information that calls into question the conclusions of the original safety assessment.

II. Literatures and References

A. Documentation regarding the approval and consumption of the GMO in other countries.

B. Any other information to demonstrate safety of the GMO as food.

C. For safety assessment of unintended effects, refer to Annex VI.

Annex II - Information Required for the Genetically Modified Plants with Stacked Traits

I. Applicable objects to foods produced using genetically modified plants with stacked traits obtained through conventional breeding between genetically modified plants that are already approved.

II. Classification of genetically modified plants with stacked traits:

A. Category I: plants with two or more unrelated traits.

B. Category II: plants with two or more related traits, but with different mechanisms of action.

C. Category III: plants with two or more traits that function in the same biosynthetic pathway.

III. Food safety assessment of the genetically modified plants with stacked traits belonging to category I and II

A. The following bridging studies would need to be carried out to confirm that the stacked product is derived from the combination by traditional breeding of the single genetically modified events.

A) molecular characterization: a fingerprint-type Southern blot analysis, in comparison to the parental lines;

B) information on gene expression: analysis of gene expression in the stacked product to confirm that gene expression in relevant tissue(s) is comparable to that in the single genetically modified events; and

C) compositional analysis: the compositional analysis for the stacked product would be undertaken over a single growing season (4 sites), and relevant comparisons would be made either with the single-event genetically modified plants or with the non-genetically modified control of comparable genetic background.

B. Protein safety evaluation and animal feeding studies would need to be conducted when necessary.

A) If no changes to protein mode of action can be expected in the stacked product, no additional safety evaluation of the proteins and animal feeding studies would be required.

B) If an interaction between the introduced proteins affecting their mode of action is expected, the need for additional studies should be evaluated on a case-by-case basis.

IV. Genetically modified plants with stacked traits belonging to category III are considered as new genetically modified foods. A complete food safety assessment carried out in accordance with Annex I is required.

V. Review process

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

ANNEX III – Information Required for the Genetically Modified Microorganisms

I. Application materials must include the following information

A. Description of the genetically modified microorganism

A) Description of the bacterial, yeast, or fungal strain;

B) Description of the food produced using the genetically modified microorganisms;

C) Information about the preservation and identification of the genetically modified microorganisms used in food production or contained in food

B. Description of the host microorganism and its use as food

The data and information should include, but need not be restricted to:

A) Identity

a. scientific name, common name or other name(s) used to reference the microorganism;

b. strain designation;

c. information about the strain and its source, or accession numbers or other information from a recognized culture repository from which the organism or its antecedents may be obtained; and

d. if applicable, information supporting its taxonomical assignment.

B) History of use and cultivation, known information about strain development (including isolation of mutations or antecedent strains used in strain construction);

C) Information on the recipient microorganism's and related species' genotype and phenotype relevant to safety, including

a. any known toxins, antibiotics, antibiotic resistance factors or other factors related to pathogenicity, or immunological impact;

b. the genetic stability of the microorganism including, as appropriate, the presence of mobile DNA elements, i.e. insertion sequences, transposons, plasmids, and

prophages.

D) History of safe use in food production or safe consumption in food, which may include information on

a. For microorganism which does not include in the EFSA Qualified Presumption of Safety (QPS) list or generally recognized as safe (GRAS) by the United States Food and Drug Administration (FDA) or with a history of safe use in food, information relating to toxigenicity and pathogenicity for humans should be provided.

b. how the recipient microorganism is typically grown;

c. how the recipient microorganism is typically transported;

d. how the recipient microorganism is typically stored;

e. quality assurance, including those to verify strain identity and production specifications for microorganisms and foods; and

f. whether these organisms remain viable in the processed food or are removed or rendered non-viable as a consequence of processing.

E) Information on the relevant production parameters used to culture the recipient microorganism.

C. Description of the donor organism(s)

A) Identity.

a. Scientific name, common name or other name(s) used to reference the organism;

b. strain designation;

c. information about the strain and its source, or accession numbers or other information from a recognized culture repository from which the organism or its antecedents may be obtained; and

d. if applicable, information supporting its taxonomic assignment; its usual or common name, scientific name and taxonomic classification;

B) information about the organism or related organisms that concerns food safety;

C) information on the organisms genotype and phenotype

relevant to its safety including any known toxins, antibiotics, antibiotic resistance factors or other factors related to pathogenicity, or immunological impact; and

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

D. Description of the genetic modification(s)

Including vector and construct

A) The description of the strain construction process should include:

a. information on the specific method(s) used for genetic modification;

b. information on the DNA used to modify the microorganism, including

a) the source (e.g. plant, microbial, viral, synthetic);

b) identity;

c) expected function in the genetically modified microorganism; and

d) copy number for plasmids;

c. intermediate recipient organisms.

B) Information should be provided on the DNA added, inserted, deleted, or modified, including:

a. the characterization of all genetic components, including

a) marker genes;

b) vector genes;

c) regulatory elements; and

d) 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.

E. Characterization of the genetic modification(s)

A) Information should be provided on the DNA modifications in the genetically modified microorganism; this should include:

a. the characterization and description of the added, inserted, deleted, or otherwise modified genetic materials, including plasmids or other carrier DNA used to transfer desired genetic sequences. This should include

a) an analysis of the potential for mobilization of any plasmids or other genetic elements used;

b) the locations of the added, inserted, deleted, or otherwise modified genetic materials (site on a chromosomal or extrachromosomal location); and

c) if located on a multicopy plasmid, the copy number of the plasmid;

b. the number of insertion sites;

c. the organization of the modified genetic material at each insertion site including

a) the copy number and sequence data of the inserted, modified, or deleted material;

b) plasmids or carrier DNA used to transfer the desired genetic sequences; and

c) the surrounding sequences;

d. identification of any open reading frames within inserted DNA, or created by the modifications to contiguous DNA in the chromosome or in a plasmid; and

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

B) Information should be provided on any newly expressed substance(s) in the genetically modified microorganism.

This should include information listed in a to f. If the newly expressed substance(s) in the genetically modified microorganism were evaluated to be substantially equivalent to existing substances with known history of safe use as foods, no further assessment would be required.

Otherwise, assessments listed in B) of F should be conducted.

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 (intracellular, periplasmic - for Gram-negative bacteria, organellar - in eukaryotic microorganisms, secreted) in the microorganism of the expressed gene product(s), and, when applicable, the levels of its metabolites in the organism;

e. the amount of the inserted gene product(s) if the function of the expressed sequence(s)/gene(s) is to alter the level of a specific endogenous mRNA or protein; and

f. the absence of a gene product, or alterations in metabolites related to gene products, if applicable to the intended function(s) of the genetic modification(s).

C) In addition, information should be provided:

a. to demonstrate whether the arrangement of the modified genetic material has been conserved or whether significant rearrangements have occurred after introduction to the cell and propagation of the recombinant strain to the extent needed for its use(s) in food production, including those that may occur during its storage;

b. to demonstrate whether deliberate modifications made to the amino acid sequence of the expressed protein result in changes in its post-translational modification or affect sites critical for its structure or function;

c. to demonstrate whether the intended effect of the modification has been achieved and that all expressed traits are expressed and inherited in a manner that is stable for the extent of propagation needed for its use(s) in food production and is consistent with laws of inheritance. It may be necessary to examine the inheritance of the inserted or modified DNA or the expression of the corresponding RNA if the phenotypic

characteristics cannot be measured directly;

d. to demonstrate whether the newly expressed trait(s) is expressed as expected and targeted to the appropriate cellular location or is secreted in a manner and at levels that is consistent with the associated regulatory sequences driving the expression of the corresponding gene;

e. to indicate whether there is any evidence to suggest that one or more genes in the recipient microorganism has been affected by the modifications or the genetic exchange process; and

f. to confirm the identity and expression pattern of any new fusion proteins.

F. Safety assessment

A) Compositional analyses of key components

a. Analyses should be performed by comparing the concentrations of key components of foods produced by genetically modified microorganisms to a conventional counterpart produced under the same conditions using an equivalent analysis.

b. Ideally, the comparator(s) used in this assessment should be food produced using the near isogenic parent strain.

B) Initial assessment of possible toxicity and allergenicity of newly expressed substances in genetically modified microorganisms

a. For initial assessment of possible toxicity of the newly expressed substances, the following information should be provided.

a) Function and concentration in the food;

b) the expected exposure, possible intake, and possible effects on diets;

c) history of safe use as food;

d) information should be provided to ensure that genes coding for known toxins or anti-nutrients present in the donor organisms are not transferred to genetically modified microorganisms;

e) in the case of proteins, the assessment of potential toxicity should focus on

1. amino acid sequence similarity between the protein and known protein toxins and anti-nutrients (e.g. protease inhibitors, siderophores);
2. stability to heat or processing; and
3. stability to degradation in appropriate representative gastrointestinal model systems.
4. Appropriate oral toxicity studies (Annex IV) may be carried out in cases where the proteins present in the food show no close similarity to proteins that have been safely consumed in food, nor has been consumed safely in food previously, taking into account its biological function in microorganisms where known.

b. For initial assessment of possible allergenicity of the newly expressed proteins, the following information should be provided.

a) The source of the introduced protein

1. Information should describe any reports of allergenicity associated with the donor organism.
2. When evidence of IgE mediated oral, respiratory or contact allergy of the donor organism is available, the following information may be provided.

1) The availability of sera for screening purposes;

2) documented type, severity and frequency of allergic reactions;

3) structural characteristics and amino acid sequence of the known allergenic proteins from that source; and

4) if available, physicochemical and immunological properties of the known allergenic proteins from the source.

3. Genes derived from known allergenic sources

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

4. When genes are derived from non-allergenic sources, the following information should be provided.

    1) Level and location of the newly expressed protein; and

    2) function of the newly expressed protein.

b) Amino acid sequence homology

1. Result on sequence homology searches comparing the structure of all newly expressed proteins with all known allergens must be provided. Searches should be conducted using various algorithms to predict overall structural similarities.

    1) Strategies such as stepwise contiguous identical amino acid segment searches must be performed for identifying sequences that may represent linear epitopes.

    2) The newly expressed protein is considered significantly similar to a known allergen when more than 35% identity in a segment of 80 or more amino acids, contains linear epitope(s) or complies with other scientifically justified criteria, the sequence alignment result is positive.

2. 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, if available, from individuals allergic to the

identified allergenic source. If there is no available serum, other scientifically sound methods and tools may be used to assess the allergenicity potential of the newly expressed protein(s).

c) Pepsin resistance

1. The assessment should be conducted with a consistent and well-validated pepsin degradation protocol.

2. Further analyses are required for a newly expressed protein with a pepsin digestion fragment larger than 3.5 kDa.

3. Alternative protocols may be used where adequate justification is provided.

c. For proteins from sources not known to be allergenic, and which do not exhibit sequence homology to a known allergen, targeted serum screening or other related IgE serum screenings and immunological tests might be considered.

d. Initial assessment of possible toxicity and/or allergenicity of the newly expressed substances in genetically modified microorganisms may require the isolation of the new substance from the genetically modified microorganism, 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 genetically modified microorganism.

e. When the results of the initial assessment indicate that the newly expressed substance is possible toxin(s) and/or allergen(s), further assessment should be conducted according to Annex IV and/or V.

C) Evaluation of metabolites

Some genetically modified microorganisms may have been modified in a manner that could result in new or altered levels of various metabolites in the food.

a. 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.

b. New or altered levels of metabolites produced by a genetically modified microorganism may change the population of microorganisms in mixed culture, potentially increasing the risk for growth of harmful organisms or accumulation of harmful substances. Possible effects of genetic modification of a microorganism on other microorganisms should be assessed when a mixed culture of microorganisms is used for food processing.

D) Nutritional modification

a. Foods derived from genetically modified microorganisms 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.

b. The likely intake of the food derived from the genetically modified microorganism should be estimated. 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.

c. Although the genetically modified microorganism components may be individually assessed as safe, the impact of the change on the overall nutrient profile should be determined.

d. 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 genetically modified microorganism) as appropriate comparators to assess the nutritional impact of the food.

e. It is also important to ascertain to what extent the modified nutrient is bioavailable and remains stable with time, processing and storage.

f. Animal feeding studies may be warranted for foods derived from genetically modified microorganisms if changes in the bioavailability of nutrients are expected or if the composition is not comparable to conventional foods.

g. Foods designed for health benefits, may require an assessment beyond the scope of these guidelines such as specific nutritional, toxicological or other appropriate studies.

h. In consideration of geographical and cultural variation in food consumption patterns, the nutrient and the populations affected should be identified for nutritional changes to a specific food.

i. 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.

j. 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.

E) Antibiotic resistance and gene transfer

a. Antibiotic resistance

a) When the microorganisms contain antibiotic resistance genes, describe the location of the genes in the cell;

b) any indication of the presence of plasmids, transposons, and integrons containing such resistance genes should be specifically addressed.

b. Gene transfer

a) Note the location of the genetic material to be inserted into the genome. Chromosomal integration of the inserted genetic material may be preferable to localization on a plasmid;

b) where the genetically modified microorganism will remain viable in the gastrointestinal tract, genes should be avoided in the genetic construct that could provide a selective advantage to

recipient organisms to which the genetic material is unintentionally transferred; and

c) sequences that mediate integration into other genomes should be avoided in constructing the introduced genetic material.

F) Viability and residence of microorganisms in the human gastrointestinal tract

For applications in which genetically modified microorganisms used in production remain viable in the final food product, the following information must be provided:

a. Demonstration of the viability or residence time of the microorganism alone and within the respective food matrix in the digestive tract.

b. Assessments of the impact on the intestinal microflora in appropriate system.

G) Food processing

The potential effects of food processing, including home preparation, on foods derived from genetically modified microorganisms should be considered.

G. Other considerations

The safety assessment should be reviewed in the light of new scientific information that calls into question the conclusions of the original safety assessment.

II. Literatures and references

A. Documentation regarding the approval and consumption of the GMO in other countries.

B. Any other information to demonstrate safety of the GMO as food.

C. For safety assessment of unintended effects, refer to Annex VI.

Annex IV - Safety Assessment of Possible Toxicity

I. Current dietary exposure and possible effects on population sub-groups should be considered.

II. Appropriate oral toxicity studies 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 organism where known.

III. 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 organism 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.

IV. Assessment of possible toxicity of the newly expressed substances in GMO may require the isolation of the new substance from the GMO, 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 GMO.

Annex V- Safety Assessment of Possible Allergenicity

I. Serum screening

A. For those proteins that originate from a source known to be allergenic, or have sequence homology with a known allergen, specific serum screenings should be performed where sera are available.

A) 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. A major allergen is defined as one to which more than 50% of individuals sensitive to that substance react in IgE-specific immunoassays.

B) a minimum of 24 relevant sera is required to achieve the same level of certainty in the case of a minor allergen. A minor allergen is defined as one to which more than 20% but less than 50% of individuals sensitive to that substance react in IgE-specific immunoassays.

B. 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. A positive result in such tests would indicate a potential allergen.

C. Assessment of possible allergenicity of the newly expressed proteins in GMO may require the isolation of the new proteins from the GMO, or the synthesis or production of the proteins from an alternative source, in which case the material should be shown to be biochemically, structurally, and functionally equivalent to that produced in the GMO.

II. Other considerations

A. The types of food processing would be applied and its effects on the presence of the protein in the final food product; and

B. Other scientifically sound methods and tools may be considered in assessing the allergenicity potential of the newly expressed proteins.

Annex VI - Safety Assessment of Unintended Effects

Safety assessment of unintended effects should include data and information on the insertion of DNA sequences, subsequent conventional breeding of the GMO, and/or the random insertion of DNA sequences into the organism genome which may cause disruption or silencing of existing genes, activation of silent genes, or modifications in the expression of existing genes.

Comparative safety assessment process on GMO can be applied to identify, if any, unintended effects.

I. Provide the following information:
   A. the phenotypic characteristics of the organism; and
   B. 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.

II. The biological relevance and potential effects on food safety of unintended effects should be evaluated.