

Standards for the Safety Assessment of Genetically Modified Foods (Seed Plants)

Chapter 1 General Provisions

Section 1 Background

Based on the “Guideline for the Safety Assessment of Foods and Food Additives Produced by Recombinant DNA Techniques” issued in 1991 by the Ministry of Health and Welfare, the first safety assessments of food additives produced by recombinant DNA techniques and of genetically modified foods derived from seed plants were conducted in 1994 and 1996, respectively. Since then, safety has been confirmed for a number of genetically modified foods and food additives. The safety assessment of genetically modified foods has become mandatory since April 2001, following a revision of the standards for foods and food additives under the provisions of the Food Sanitation Law.

On the other hand, internationally, the Codex Alimentarius Commission has authorized the two guidelines “Principles for the risk analysis of foods derived from modern biotechnology” and “Guideline for the conduct of food safety assessment of foods derived from recombinant-DNA plants” in July 2003.

Also, in July 2003, the Food Safety Commission was founded to conduct safety assessments of foods including genetically modified foods and food additives at the request of the Ministry of Health, Labor and Welfare. The standards stated herein include the principles, basic concepts and standards required for the safety assessment of genetically modified foods (seed plants) to be conducted by the Food Safety Commission, and these have been composed based on the former and Codex guidelines.

Section 2 Definitions

1 Recombinant DNA techniques

Technique that recombinant DNA molecules prepared by cleavage and recombination of DNA using enzymes or other methods are transferred to living cells for proliferation (the term refers to the techniques that overcome natural physiological reproductive or recombinant barriers and that are not techniques used in traditional breeding and selection).

- 2 Host
A living cell or individual organism into which DNA is transferred through recombinant DNA techniques
- 3 Vector
A carrier DNA that transfers the target genes or DNA fragment into the host for its proliferation or gene expression
- 4 Inserted gene
A gene inserted into the vector
- 5 Inserted DNA
A DNA inserted into the vector
- 6 Donor
A microbe, animal or plant that supplies the inserted DNA
- 7 Expression vector
A final vector carrying an inserted gene/DNA constructed to confer a new trait(s)
- 8 Recombinant plant
A host containing recombinant DNA
- 9 Gene product
The RNA or protein deduced from the DNA sequence of the inserted gene
- 10 Genetically modified foods (seed plants)
Foods derived from seed plants produced by using recombinant DNA techniques

Section 3 Scope and objective

The purpose of this document is to provide the standards required for the safety assessment of genetically modified foods (seed plants). This document does not intend to address environmental, ethical, moral and socio-economic aspects of the research and development, production, and marketing of the genetically modified foods (seed plants).

Section 4 Principles and basic concepts for safety assessment of genetically modified foods (seed plants)

As for genetically modified foods (seed plants), not only their direct harmful effects on human health but also the nutritional consequences of their long-term consumption should be considered in their safety assessment. Most foods that we eat today are known to be substantially harmless during our long history of use, or to become harmless when cooked or processed. For the foods obtained through traditional breeding techniques, toxicological or nutritional testing has not been required because we know from experience that, in most cases, breeding does not

cause significant changes in traits that may affect the human health. In general, applying traditional toxicological tests using animals for risk assessment to whole foods is not common due to their great technical difficulties. Meanwhile, safety has not necessarily been confirmed for every component in the foods. Therefore, the safety or the absence of significant health effects of most foods has been confirmed through their empirical uses as whole foods.

Also in the safety assessment of genetically modified foods (seed plants), it is difficult to scientifically confirm the safety of each component. Currently, it is therefore reasonable to conduct safety assessment by comparison of genetically modified foods with conventional foods regarding the traits that have been intentionally or unintentionally added or eliminated. Unintended changes are not limited to recombinant DNA techniques but are also associated with traditional breeding. However, the evaluation and prediction of these unintended changes are important in the safety assessment of genetically modified (recombinants) plants as food. For the new technology without a long history of safe use, potential risks of inducing drastic changes in harmful components or newly generating toxic proteins through the unintended changes in traits should be excluded beforehand as much as possible.

Safety assessment is feasible only when changes in the properties of genetically modified foods (seed plants) are scientifically predictable from the properties of the inserted DNA (gene) and the changes in the modified genome, and when sufficient comparison can be conducted between the host and recombinant plants.

With the above-mentioned principles, safety assessment should be conducted based on the following basic concepts.

- 1 The safety assessment of genetically modified foods (seed plants) is feasible only when they can be compared with hosts or conventional varieties with a long history use as foods, or existing foods. This is based on the considerations that safeties on the existing traits, aside from those that have been added by genetic modifications, has been widely accepted and requires no further assessments, or that sufficient findings for their assessment has already accumulated.
- 2 The most critical elements to be considered in safety assessment are the effects on human health of the traits that have been intentionally added, altered or eliminated through recombinant-DNA techniques, and of the risks such as the production of new harmful components and changes in major nutrients. Furthermore, where recombinant-DNA techniques have been used to intentionally develop the recombinant plants with altered contents of nutrients, functional components or

harmful components, the safety of these modifications on human health should be confirmed considering the contents and consumption of such components in other foods.

- 3 The safety assessment of genetically modified foods (seed plants) will be conducted in terms of all changes in the traits expected to be added to the seed plants. For instance, inserting a DNA sequence not only confers a specific trait to the plant (intended effect) but also may also confer additional traits or eliminate or modify the existing traits of the host (unintended effects). These unintended effects may be harmful, beneficial or neither harmful nor beneficial to the growth of the host plant or for the safety aspects of the genetically modified foods. Nevertheless, the effects of the intended and unintended addition of traits or changes in traits should be individually assessed from toxicological and nutritional viewpoints, while the assessment of the safety of the foods should also be conducted from the global viewpoint. For this safety assessment, sufficient data or information should be available to minimize the risk of unpredicted adverse effects on human health caused by genetically modified foods (seed plants).
- 4 For genetically modified foods (seed plants), the potential effects of food processing, including home cooking, should also be considered. For example, alterations could occur in the heat stability of an endogenous toxicant or the bioavailability of an important nutrient after processing. Therefore, information should be provided on the processing conditions used in the production from the plant and changes in the food ingredients. For example, in the case of vegetable oil, information should be provided on the extraction process and any subsequent purifying steps.
- 5 Some recombinant plants may exhibit traits (e.g. herbicide tolerance) which may indirectly cause accumulation of pesticide residues, altered metabolites of such residues, toxic metabolites, contaminants, or other substances which may be relevant to human health. These possibilities should also be considered in the safety assessment.
- 6 In the safety assessment, all the edible parts of the seed plant should be considered. For example, in products such as rapeseed oil, the extracted material from the recombinant plant is usually consumed, but other parts may be also eaten. In such cases, safety assessment of the recombinant plant itself should be conducted considering these points.
- 7 Studies intended to obtain data for safety assessment should be designed and conducted with sound scientific concepts and principles, as well as, where appropriate, Good Laboratory Practice (GLP). Raw data should be submitted upon

request. Data or information necessary for safety assessment, such as experimental data obtained by the developers, published scientific papers and information provided by third parties, should have been obtained using sound scientific methods and analyzed by using appropriate statistical methods. Whenever possible, the sensitivity of all analytical methods should be documented.

- 8 For the safety assessment, it may be required to isolate the novel substance produced in the recombinant plant, or to synthesize or produce the substance from an alternative source. In this case, the material should be shown to be biochemically, structurally, and functionally equivalent to that produced in the recombinant plant.
- 9 The safety assessment of the currently used antibiotic resistance markers such as the kanamycin-resistance gene has been appropriately conducted, and there have been no safety concerns to date. However, in the future development, alternative transformation methods that do not result in any residual antibiotic resistance genes in food should be considered to use, where such techniques are available and demonstrated to be safe.
- 10 Along with the continuing progress in the recombinant DNA technology, these standards should be revised as required.

Chapter 2 Standard for the Safety Assessment of Recombinant Seed Plants as Foods [Genetically Modified Foods (Seed Plants)]

Section 1 Properties of the host used as a counterpart for comparison (or other comparator) in safety assessment and its differences from the recombinant plant

Information on the following items, 1-5, should be outlined to demonstrate that the genetically modified food (seed plant) can be compared with an existing host or other comparator for its safety assessment. The differences between the recombinant plant and the host or other comparator described in 6 should be clearly identified.

1 Host and introduced DNA

- (1) Species (and subspecies, variety and strain, as necessary) and origin of the host
- (2) Species (and subspecies, variety and strain, as necessary) and origin of the donor
- (3) Properties of the introduced DNA and methods for DNA transfer

2 History of utilization of the host as food

3 Components of the food derived from the host

- (1) Outline of the types and amounts of major nutrients (proteins, lipids, etc.) contained in the edible parts of the host.
- (2) Outline of the types and amounts of toxic constituents and anti-nutrients (i.e.

substances that inhibit digestion and/or absorption of nutrients, such as trypsin inhibitor and phytic acid) contained in the host.

- 4 Usages of the host and recombinant plants as food and their differences
 - (1) Harvesting time (level of ripeness) and storage method
 - (2) Consumable (edible) parts
 - (3) Consumption volume
 - (4) Cooking and processing methods
- 5 Reasons for using any plant/product in addition to the host as a comparator and its properties as food
- 6 Differences that need to be assessed for safety

If there is any existing host (and other comparator) which can be compared with the genetically modified food (seed plant), comparative assessment should be performed regarding the criteria listed in the following sections.

Section 2 Purposes and usages of the recombinant plant

The purposes and usages of the recombinant plant should be clarified.

Section 3 Host

- 1 Taxonomic status (species, variety, and strain)

The species (scientific name), variety and strain of the host should be identified. Its broad history of safe consumption as food and dietary customs in the world should be documented.
- 2 Genetic ancestry and breeding history

It should be described whether or not the genetic ancestry of the host is capable of producing harmful physiological substances such as toxins and anti-nutrients. If any ancestral plant produces a harmful substance, the breeding process employed to reduce or eliminate the harmful substance should be described as much as possible.
- 3 Production of harmful physiological substances

The name, mechanism of activity and amount of any harmful physiological substance produced by the host should be described.
- 4 Allergenicity

Any findings regarding the allergenicity (including the induction of gluten sensitive enteropathy) of the host used for developing the recombinant plant should be reported.
- 5 Foreign pathogenic factors (e.g. viruses)

If any pathogen is known to be capable of infecting the host plant used to develop the genetically modified food (seed plant), the pathogen should not be capable of

infecting humans or should not have any gene exerting pathogenicity to humans.

6 Safe consumption

Where any technique or processing has been utilized to ensure the safe consumption of the host used to develop the recombinant plant, it should be noted (as in the cases of beans containing cyanogenic compounds).

7 Close relatives of the host

If there is any genetically close plant species that produces a harmful physiological substance, it should be determined whether or not the harmful substance is produced in the recombinant plant. If the recombinant plant produces the substance, its safety should be demonstrated based on scientific rationales including its daily intake.

Section 4 Vector

1 Name and origin

The name and origin of the vector (e.g. plasmid) used for gene transfer should be described.

2 Properties

(1) Size and sequence of the vector DNA

The number of base pairs and nucleotide sequence of the DNA should be identified. If the sequence is deposited into public databases, the accession number should be noted.

(2) Restriction map

The restriction site map of the vector should be provided. The names of the restriction endonucleases used and the number and size of DNA fragments should be indicated.

(3) The absence of any nucleotide sequence to produce a known harmful product

The vector should not harbor any nucleotide sequence known to produce a harmful protein.

(4) The properties of any drug (including antibiotics)-resistant gene on the vector should be identified.

(5) Transmissibility

In principle, the vector should not show any transmissibility (i.e., the potential to autonomously migrate from the host to other organisms). If the vector is transmissible, information on the range of target organisms should be provided.

Section 5 Inserted DNA, gene products and expression vector construction

1 Donor of the inserted DNA

(1) Name, origin and taxonomy

The name, origin and taxonomic status of the donor should be described.

(2) Safety

- The donor of the inserted DNA should not show any pathogenicity to humans or produce toxins. Moreover, if any pathogenic strain is known within the donor species, as in the case of *E. coli*, it should be indicated that the donor has been derived from a non-pathogenic strain.
- If the donor has been reported to be pathogenic or to produce a toxin, it should be demonstrated that the inserted DNA itself does not commit to produce any toxin, and that the protein(s) derived from the inserted DNA is non-pathogenic.
- It should be clear whether the donor of the inserted gene has a history of safe consumption.

2 Properties of the inserted DNA or genes (including the drug-resistance gene) and their gene products

(1) Methods for cloning or synthesis of the inserted gene

Methods used for cloning or synthesizing the inserted gene should be described.

(2) Size, nucleotide sequence, and restriction map

The number of base pairs and nucleotide sequence of the DNA fragment aimed to be introduced into the host should be clarified. The restriction site map should be provided with the names of the restriction endonucleases and the number and sizes of DNA fragments.

(3) Function of the inserted gene

The function of the inserted gene and the properties and functions of its products (RNA and protein) should be clarified. Scientific rationales should be presented to demonstrate that the protein has no adverse effects. These are also applied to cleaved or digested proteins within the plant cell following transcription and translation of the inserted gene. The method and results of the sequence homology search between the protein produced by the inserted gene and the known toxic proteins should be described. In principle, the product protein should not have significant homology. If it shows any significant homology, scientific rationales should be presented to demonstrate that it does not affect human health.

(4) Antibiotic resistance gene

- The administration routes (oral, intravenous, etc.) of the antibiotic should be described.

- The mechanism of resistance should be described.
 - The safety of the metabolite associated with the resistance should be demonstrated.
 - The usages (regimen, amount, purpose, etc.) of the antibiotic should be described.
- 3 Regulatory regions involved in the expression of the inserted genes (including the drug-resistance gene)
- (1) Promoter
The origin and properties of the promoter used should be described.
 - (2) Terminator
The origin and properties of the terminator used should be described.
 - (3) Others
The origin and properties of other nucleotide sequences inserted to regulate the expression of the inserted gene should be described.
- 4 Methods for construction of the expression vector with foreign DNA inserts
The methods for insertion of the foreign DNAs into the vector should be presented.
- The methods used to construct the expression vector to be introduced into the host. Especially, when the vector has been constructed by connecting two or more genes or fragments, these construction methods should be described.
 - The procedures by which the promoter, open reading frame, terminator and drug (including antibiotics)-resistance gene were introduced into the vector should be clearly described.
- 5 Expression vector constructed
- (1) Size, nucleotide sequence and restriction map
The number of base pairs and sequence of the inserted DNA in the expression vector constructed should be identified. The restriction site map should be provided with the names of the restriction endonucleases and the number and sizes of DNA fragments.
 - (2) In principle, the expression vector finally introduced into the host should not contain any open reading frame that can express an unintended protein within the recombinant plant.
If the vector contains any gene capable of expressing an unintended protein in the recombinant plant, scientific rationales should be presented to demonstrate the safety of the gene and its product.

- (3) In the expression vector used for introducing genes into the host, the region intended to be introduced should be clearly indicated.
- (4) The expression vector to be introduced into the host should not be contaminated by any unintended genes.

6 Methods for DNA transfer into the host and subsequent breeding

The methods used for introduction of the DNA into the host plant should be presented. Specifically,

- the method used to introduce the DNA into the host,
- the selection method (the method used for selecting the host holding the introduced DNA(s)), and
- the method used for regenerating the plants should be described.

The strain(s) subject to the safety assessment should be specified, for example, with a tree-diagram showing the breeding process.

Section 6 Recombinant plant

1 Inserted genes

(1) Copy number and flanking sequences of the host genome

The nucleotide sequence, size and origin of the DNA(s) inserted into the host genome should be identified.

The structure and copy number of the DNA(s) inserted into the host genome (e.g. the copy number and status of inserted DNA(s), including the presence or absence of deletion or duplication in the inserted gene, and the insertion site in the host genome) should be identified.

The nucleotide sequence data of the inserted DNA and of the flanking regions of the host genome should be identified. Wherever possible, it should be demonstrated that the insertion does not cause any alterations in the nucleotide sequences of the host genes. If the sequence of a host gene has altered, it should be demonstrated that the alteration is not a safety concern.

(2) Presence of open reading frames and the possibility of their transcription and expression

- In principle, scientific data should be presented to demonstrate that the DNA(s) inserted into the host genome contains no open reading frame that expresses an unintended protein. Any alterations in the open reading frame caused by mutations, deletions or rearrangements occurring during the DNA

insertion event should be identified. Any possibility that the DNA insertion leads to expression of an unintended protein excluded, e.g. by Northern blotting or RT-PCR.

- If any open reading frame that can express an unintended protein has been identified, scientific rationales should be presented to demonstrate that the gene and its product protein do not affect human health.

2 Spatial and temporal expression levels of the gene product in the recombinant plant

The method for quantification of the gene product(s) derived from the inserted gene(s) (including the antibiotic resistance gene) should be available, and the timing and levels of its expression should be identified in the tissues or organs of the recombinant plant.

Any changes in the tissues specificity, timing, and levels of expression in the recombinant plant should be reviewed to demonstrate the safety of the changes.

3 Daily intake of the gene product (protein) and its significance

The ratio of the daily intake of the gene product to that of the total protein in humans should be estimated. In principle, the intake of the product protein should not account for a significant portion of the daily total protein intake. If it is significant, scientific rationales should be presented to demonstrate that it does not affect human health.

- If an antibiotic resistance gene is used, scientific rationales should be presented to demonstrate the absence of any problem associated with inactivation of the antibiotic, based on the amount of consumption of the expressed protein (the enzyme catalyzing the antibiotic), and the digestion by artificial gastric or intestinal fluid and reduction by cooking (e.g. heating) described in Section 6-4-(3).

4 Allergenicity of the gene product (protein) (including the drug-resistance gene)

The safety of the gene product should be confirmed through reviewing the following items (1) to (4). If the safety is not confirmed by the information on items (1) to (4), the confirmation should be made by judging the safety from the global viewpoint with the information on item (5) in addition to items (1) to (4). All the items are not necessarily mandatory when appropriate scientific rationales are provided.

(1) Allergenicity of the donor of the introduced gene

Information on the allergenicity (hereafter including the induction of gluten-sensitive enteropathy) of the donor of the introduced gene (including the drug resistance gene) should be described.

(2) Allergenicity of the gene product (protein)

Information on the allergenicity of the gene product (protein) itself should be described.

(3) Sensitivity of the gene product (protein) to physicochemical treatments

Data on the alterations in the molecular weight, enzymatic activity and immunoreactivity of the gene product (protein) by the following treatments, to , should be provided. The molecular weight should be determined by SDS polyacrylamide gel electrophoresis. The immunoreactivity should be determined by Western blotting, ELISA or an equivalent method using the polyclonal antibody rose against the undenatured gene product (protein).

Acidic and enzymatic (pepsin) treatment with artificial gastric fluid

Alkaline and enzymatic (pancreatin) treatment with artificial intestinal fluid

Heat treatment under similar conditions to those used for usual cooking or processing of foods.

(4) Similarity of amino acid sequence of the gene product (protein) with known allergens (including the proteins involved in gluten-sensitive enteropathy)

The primary structure of the gene product (protein) should show no sequence homology with any known allergen (Amino acid sequence homology searches should be performed to survey the presence of any sequence that can be a possible antigenic determinant (epitope)). The name of the allergen database used and the conditions, methods, and results of the searching performed should be presented.

(5) IgE-binding activity of the gene product (protein)

If the safety of the gene product on human health cannot be confirmed by items (1) to (4), the IgE-binding activity of the gene product (protein) should be assessed.

The sera of allergic patients to be used should be selected according to the following to .

In cases where the donor of the introduced gene has allergenicity, sera with high titers of donor-specific IgE should be used.

In cases where the gene product has sequence homology with a known allergen, sera with high titers of IgE specific for the organism bearing the

allergen should be used,

In cases where the gene product have no sequence homology with any known allergen, but its allergenicity cannot be completely denied by the information on items (1) to (3), sera with high titers of IgE specific for an organism closely related to the gene donor should be used,

In cases where appropriate sera for to are not available, sera with high titers of IgE specific for major food allergens (egg, milk, soy bean, rice, wheat, buckwheat, cod, shrimp and peanut) should be used.

In cases where the donor of the introduced gene has allergenicity, and its safety cannot be convincingly confirmed regardless of the negative results obtained in the IgE binding assay using the sera of patients allergic to the gene product (protein), data obtained from clinical tests (e.g. skin tests, oral loading tests) are needed.

5 Stability of the gene introduced into the recombinant plant

- Cultivation tests should be performed over a sufficient number of generations, and the stability of the structure of the introduced gene(s) and tissue or organ specificity and levels of its expression should be confirmed by Southern and Western blottings or other methods.
- The recombinant plants subjected to the tests, i.e. which line and generation of plants in the breeding process described in Section 5 “6 Methods for DNA transfer into the host and subsequent breeding” were used, should be specified.
- The trait conferred by the introduced gene and the expression levels of the gene should be monitored over several generations to confirm the stability of the structure and copy number of the introduced gene(s).

6 Effect of the expressed gene product (protein) on the metabolic pathways (including its potential to react with substrates in the host)

If the gene product is an enzyme, its substrate specificity should be determined. In principle, the substrate specificity of the enzyme should be high. It should be demonstrated that the substrate specificity has not been changed through the gene insertion event. If the substrate specificity is low or has been changed, scientific rationales should be presented to demonstrate that it does not affect human health.

In addition, if the gene product interacts with a metabolic pathway in the host as an enzyme and causes alterations in the related components of the pathway, scientific rationales should be presented to demonstrate that the alterations do not

affect human health.

7 Differences from the host

Based on comparative data between the recombinant plants and non-recombinant plants including the host, it should be determined whether or not the recombinant plant is significantly different from the host in terms of nutritional components, toxins and anti-nutrients. In principle, there should be no significant differences. If any significant differences are revealed, scientific rationales should be presented to demonstrate that the compositional difference do not affect human health. If there are any differences in the allergenic components between the recombinant and the host, the influence of such differences on allergenicity should be reviewed.

8 Approval and usage of the recombinant plant as food in other countries

Information on the approvals of the recombinant plant as food by foreign authorities and its utilization as food products should be described.

9 Methods for cultivation

- Information on how different the recombinant plant is from the host in terms of cultivation methods should be described. In principle, there should be little difference. If there is any difference, scientific rationales should be presented to demonstrate the safety of the recombinant plant.
- Usages of agricultural chemicals (e.g., pesticides and herbicides) should be described.
- In cases where the recombinant plant is tolerant to a herbicide by catalyzing it, its metabolites should be identified, and the safety of the major metabolite should be confirmed.

10 Methods for seed production and management

Any difference between the recombinant plant and the host in terms of seed production and management (e.g., preservation) should be described. In principle, there should be no difference. If there is any difference, scientific rationales should be presented to demonstrate that it is not a safety concern. Furthermore, the seeds of the host plant and of the recombinant plant at each generation after the recombination event should be stored.

Section 7 Studies required additionally when safety cannot be confirmed based on

Sections 2 to 6

The safety of the recombinant plant as food should be confirmed based on results of an appropriate study among the following list.

- (1) Acute toxicity study
- (2) Subacute toxicity study
- (3) Chronic toxicity study
- (4) Reproduction study
- (5) Mutagenicity study
- (6) Carcinogenicity study
- (7) Other required studies (e.g. intestinal toxicity, immunotoxicity, neurotoxicity, and nutritional studies)

Note: This English-translated version of "Standards for the Safety Assessment of Genetically Modified Foods (Seed Plants)" was issued in 2004 to meet the needs of non-Japanese speaking people and revised to improve readability in 2007. In the case of any discrepancy between the Japanese original and the English translation, the former will take priority.