Provisional Translation

The Food Safety Commission

Final decision on June 26, 2008

Standards for the Safety Assessment of Genetically Modified Foods (Microorganisms)

Chapter 1 General Provisions

Section 1 Background

Based on the "Guideline for the Safety Assessment of Foods and Food Additives Produced by Recombinant DNA Technologies" issued by the Ministry of Health and Welfare, the first safety assessments of food additives produced by recombinant DNA technology and of genetically modified foods derived from seed plants were conducted in 1994 and 1996, respectively. Since then, confirmation of the safety of a number of genetically modified foods and food additives has been carried out. 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 produced using recombinant-DNA microorganisms" in July 2003. Moreover, in July 2003, the Food Safety Commission was newly established, and subsequently, safety assessments of genetically modified foods and food additives have been conducted by the Food Safety Commission 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 (microorganisms) (GMFMs) 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 technology

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

2. Host

   A living cell and/or individual organism into which DNA may be transferred using recombinant DNA technologies

3. Vector

   A carrier DNA that transfers the gene of interest or DNA into a host and proliferates and expresses its gene.

4. Inserted gene

A gene inserted into a vector

5. Inserted DNA

   DNA inserted into a vector

6. Donor

   A microorganism, animal or plant that supplies inserted DNA

7. Expression vector (or transfer vector)

   A vector with inserted genes or DNA constructed to confer new properties.

8. Recombinant

   A host containing recombinant DNA

9. Gene product

   RNA or protein deduced from the base sequence of an inserted gene

10. Genetically modified microorganisms

    Microorganisms (bacteria, yeast, fungi) obtained by applying recombinant DNA technology

11. Genetically modified foods (microorganisms)

    Foods produced using genetically modified microorganisms

Section 3 Scope and Objective

The purpose of this document is to provide the standards required for the safety assessment of GMFMs. GMFMs refer to foods produced by using microorganisms obtained through applying recombinant DNA technologies.

GMFMs assessed according to these standards do not include GMFMs produced by the following microorganisms in principle, that fall under "cases where DNAs ultimately transferred to the host through recombinant DNA technologies finally consist of DNA only from microorganisms which belong to the same taxonomic species as the host belongs to," or "cases where living cells with gene composition equivalent to that of the recombinant exist in nature." Even in these cases, when the effects of GMFMs on human health are judged to be unclarified in both kind and extent, the effects need to be considered case by case. This document does not intend to address environmental, ethical, moral and socio-economic aspects of the research and development, production, and marketing of GMFMs.

In FSCJ, the safety assessment of food additives produced using genetically modified microorganisms is conducted according to the "Standards for Safety Assessments of Food Additives Produced Using Genetically Modified Microorganisms." The standards are, however, only applicable when genetically modified microorganisms (recombinants) are removed. The safety assessment of food additives in which recombinants used in their production remain is conducted following Chapters 2 and 3 of these standards.

Section 4 Principles and Basic Concepts for Safety Assessment of GMFMs

As for GMFMs, 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 assessments. Most of the foods produced using microorganisms, which originate from strains used since ancient times, have a long history of safe use before scientific safety assessments were applied. No systematic safety assessment prior to marketplace sales had been generally conducted on the microorganisms, which are obtained through conventional genetic alteration technology

and used in food production. New strains of bacteria, yeast, and fungi have been evaluated and selected based on their beneficial characteristics, and used in food production.

In general, applying traditional toxicological tests using animals for risk assessment to whole foods is not common due to the great technical difficulties associated with them. 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.

The appropriate risk assessment methodology must be developed on GMFMs, considering the large technical constraints in applying traditional toxicological tests using animals for risk assessment to whole foods produced using genetically modified microorganisms (GMMs). The safety assessment on GMFMs should be conducted in principle based on a comparison to conventional foods with a history of safe use, as it is on other genetically modified foods.

Needless to say, both intentional and unintentional effects need to be considered in the safety assessment. In addition to the points in the safety assessment on the other genetically modified foods, the following points are to be carefully taken into account, especially on foods derived from microorganisms: genetic stability, possibility of gene transfer, colonization of GMMs in intestinal tract, interaction between GMMs and human intestinal flora, effects of GMMs on the human intestinal system and immune system, as well as particular issues in individual food production process using GMMs.

Safety assessment is feasible only when changes in the properties of GMFMs 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 the recombinant microorganisms. Thus the safety assessment on GMFMs is first conducted on GMMs, then on final food products.

GMFMs are very diverse in their properties, uses, production processes, and other attributes. There might be cases where alive or inactivated recombinants are taken in with GMFMs. In many cases, however, recombinants are ultimately removed from foods in the production process. Thus, it is reasonable to conduct safety assessments on GMFMs on the basis of whether living recombinants remain or don't remain. Any differences in kinds and their amounts of components between GMFMs produced using the recombinant and the compared conventional food need to be clarified and be ensured that no safety issues exist. Considering the diversity in GMFMs, it might be difficult to conduct safety assessments based on the uniform standards. The additional "guidelines of the safety assessment," prepared for each product considering its production process and properties etc., may be applied for the safety assessment.

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

1. The safety assessment of GMFMs is feasible only when they can be compared with the host (conventional strains) and the food (existing foods) with a long history of use as foods. This is because the safety of the existing traits, aside from those that have been added by genetic modifications, are considered to have been widely accepted and require no further assessments, or because sufficient findings for their assessment have already been accumulated.
2. The most critical elements to be considered in safety assessment are 1) the effects on human health of the traits that have been intentionally added, altered, or eliminated through recombinant-DNA technologies, and 2) risks such as the production of new harmful components and changes in major nutrients. Furthermore, where

recombinant-DNA technologies have been used to intentionally develop GMFMs with altered contents of nutrients, functional components, or harmful components, the safety of these modifications on human health should be confirmed referring the contents and consumption of such components in other foods.

3. The safety assessment of recombinants will be conducted in terms of all changes in the traits expected to be added to the microorganisms. For instance, inserting a DNA sequence not only confers a specific trait to the microorganism (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 to the growth of the host or for the safety aspects of GMFMs. The effects of the intended and unintended addition of traits or changes in traits should be individually assessed from toxicological and nutritional viewpoints. Based on these assessments, the safety in food manufacturing use needs to be judged comprehensively. Such safety assessments require sufficient data or information to minimize the risk of unpredicted adverse effects on human health caused by recombinants.
4. In the safety assessment of recombinant used in the food manufacturing, the interactions need to be examined between the recombinant and food substrate or bacterial flora, as well as between the recombinant and human intestinal flora in cases where the recombinant remains alive in foods. In addition, the effects on immune system and intestinal system need to be considered.
5. On GMFMs, the following points also need to be considered; effects from changes in fermentation conditions; the interaction between the recombinant and other co-existing microorganisms or food matrix in the fermentation process; and resulting changes in the food ingredients. For example, in the case of yoghurt, information on fermentation conditions for the microorganism is needed.
6. The potential effects of food processing after fermentation, 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 and changes in the food ingredients.
7. Some recombinants may exhibit traits (e.g. resistance to antibiotic substances) which may indirectly cause accumulation of toxic metabolites, contaminants, or other substances which may affect human health. These possibilities should also be considered in the safety assessment.
8. Studies for obtaining data for safety assessment should be designed and be 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 determination limit of the employed analytical method should be documented.
9. Safety assessments may require isolating the novel substance produced in the recombinant, or synthesizing or producing 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.
10. 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 future development, alternative transformation methods that do not result in any residual antibiotic-resistance marker genes in food should be considered usable, where such techniques are available and demonstrated to be safe.

11. Along with the continuing progress in the recombinant DNA technology, these standards should be revised as required.

Chapter 2 Safety Assessment on GMMs (Recombinants)

Section 5 Properties of the Host which are Comparable with the Recombinant in Safety Assessment and their Differences

The outline of items 1 to 5 below should be presented to ensure that the host with safe use experience and comparable with the GMMs (recombinants) for the safety assessment, is available, and that the property of the host is characterized, as well as that the differences between the recombinant and the host are clarified.

1. Host and introduced DNA

(1) Species name (scientific name), strain name and origin of the host

(2) Species name, strain name or systemic name, and origin of the DNA donor

(3) Properties and method of DNA insertion.

2. History of use of the host in food manufacturing or as food

3. Components of the host

If harmful physiologically active substances and anti-nutrients (i.e. substances that inhibit digestion and/or absorption of nutrients) are contained in the host, an outline of the types and amounts of such substances should be provided.

4. Ways of use of the host and the recombinant in food, as well as their differences

(1) Manufacturing and storage

(2) Intended uses and patterns of use

(3) Consumption volume

(4) Cooking and processing methods

5. Differences between the recombinant and the host that need to be considered in the safety assessment

By comparing the GMMs(recombinants) with the comparable host, the safety assessment should be conducted on the items listed in Section 2 and below.

Section 6 Host

1. Taxonomic status (species name (scientific name) and strain name, etc.) and others

The scientific name and strain name, etc. should be specified. Its history of safe use as food (dietary culture) or experience of industrial use should be provided.

2. Pathogenicity and production of harmful physiologically active substances

The host should be nonpathogenic. If the host produces harmful physiologically active substances, the type, effect, and amount of such substances should be clarified.

3. Proallergic property

Any findings regarding the proallergic property of the host should be provided.

4. Parasitic and colonizing properties

Whether or not the host parasitizes or colonizes itself in humans or any other organisms should be clarified. If the host parasitizes or colonizes itself in humans or any other organisms, whether or not the host has a harmful

effect on humans or any other organisms should be clarified.

5. Foreign pathogenic factors (e.g. viruses)

The host used in the development of the recombinant should not be contaminated by foreign pathogenic factors (e.g. viruses)

6. Pathogenicity and production of harmful physiologically active substances of the host's closely related strains

If there are any pathogenic strains or any strain that may produce harmful physiologically active substances in the strains closely related to the host, whether or not such pathogenicity or production of such substances exist in the microorganisms used for the production of GMFMs should be clarified. If the production of harmful physiologically active substances is observed, scientific rationales should be presented to ensure that the food production using the microorganisms does not pose any safety issues.

Section 7 Vector

1. Name and origin

The name and origin of the vector (e.g. plasmid) used for gene transfer should be specified. The vector should not be known to be harmful to humans. In cases where DNA fragments are directly inserted to the host and inserted to the host genome through homologous recombination techniques, such procedures should be described with information on the DNA fragments (excluding items listed in Section 4).

2. Properties

(1) Number of base pairs and base sequence of the vector DNA

The number of base pairs and base sequence of the DNA should be provided. If the sequence is deposited into a public database, the accession number should be provided.

(2) Restriction map

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

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

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

(4) Drug resistance

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

(5) Transmissibility

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

(6) Host dependency

The vector used in recombination should not proliferate in any other microorganisms or in humans. If the vector proliferates in other microorganisms, information on the range of the host organisms should be provided.

Section 8 Inserted DNA, Gene Products and Expression or Transfer Vector Construction

1. Donor of the inserted DNA

(1) Name, origin and taxonomy

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

(2) Safety

- The donor of the inserted DNA should not be known to have any pathogenicity to humans or to produce any toxins. Moreover, if any pathogenic strain is known within the donor species, as in the case of *E. coli*, it should be presented to ensure 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 presented to ensure 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 clarified whether the donor of the inserted gene has a history of safe consumption.

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

(1) Methods for cloning or synthesizing the inserted gene

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

(2) Number of base pairs, base sequence and restriction map

The number of base pairs and base 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 enzymes 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 ensure that the products (protein) have no adverse effects. In particular, in cases where the gene products (protein) involve amino acid substitutions, scientific rationales should be presented to ensure that the products (protein) do not pose any safety issues.

3. Regulatory regions involved in the expression of the inserted genes and antibiotic-resistance marker genes

(1) Promoter

The origin and properties of the promoter used should be clarified.

(2) Terminator

The origin and properties of the terminator used should be clarified.

(3) If any other base sequences involved in regulation of expression of the inserted gene are integrated, its origin and properties should be clarified.

4. Methods for incorporating inserted DNA to vectors

The methods for incorporation inserted DNA to the vectors should be described, specifically the following:

(1) The methods used to construct the expression vector to be introduced into the host. These construction methods should also be described particularly, when the vector has been constructed by connecting two or more genes or fragments,

(2) The order and procedures by which the promoter, open reading frame, terminator, and antibiotic-resistance marker gene were introduced into the vector should be described.

5. Expression or transfer vector constructed

(1) Number of base pairs, base sequence, and restriction map

The number of base pairs and base sequence of the inserted DNA in the expression vector constructed should be provided. The restriction site map should be provided with the names of the restriction enzymes and the number and sizes of DNA fragments.

(2) In principle, the vector ultimately constructed should not contain any open reading frame that can express an

unintended protein within the recombinant. If the vector contains any gene capable of expressing an unintended protein in the recombinant, scientific rationales should be presented to ensure the gene and its product protein do not pose any safety issues.

(3) In the expression vector used for introducing genes into the host, the region intended to be inserted should be clearly indicated.

(4) The expression vector to be introduced into the host should be purified to prevent contamination with any unintended genes.

6. Methods of introduction of DNA into the host

The methods of introduction of the inserted DNA into the host should be described. Specifically,

- the method of introduction of the DNA into the host (if only the necessary DNA is left and the vector is ultimately removed from the recombinant through the use of homologous recombination or another technique, that method)
- the selection method (the method used for selecting the host to which the DNA is introduced).

Section 9 Recombinant

1. Gene Insertion

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

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

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 clarified.

The base sequence data of the inserted DNA and of the flanking regions of the host genome should be provided. Wherever possible, it should be presented to ensure that the insertion does not cause any alterations in the base sequences of the host genes. If the sequence of a host gene has altered, it should be presented to ensure that the alteration does not pose any safety issues.

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

In principle, scientific rationales should be presented to ensure 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 clarified. Any possibility that the DNA insertion leads to expression of an unintended protein excluded, e.g. by Northern blotting or RT-PCR, should be clarified.

If any open reading frame that can express an unintended protein has been identified, scientific rationales should be presented to ensure that the gene and its product protein do not pose any safety issues.

2. Expression levels of the gene product in the recombinant

The method for quantification of the gene product(s) derived from the inserted gene(s) (including antibiotic-resistance genes) should be available, and the levels of its expression should be provided.

Any changes in the levels of expression in the recombinant should be reviewed to demonstrate the safety of the changes.

3. Relative amount of the gene product (protein) in daily intake of protein

Assuming intakes of the foods produced using the recombinant, the amount of the daily intake of the gene product relative 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. Otherwise, scientific rationales should be presented to ensure that it does not pose any safety issues.

4. Antibiotic-resistance marker gene

When an antibiotic-resistance marker gene is used, the structure and function of said gene and its gene product should be clarified. If needed, substrate specificity should be clarified.

If it is unclear that the marker gene and its product are, during the process of food manufacturing, removed to a degree that they will pose no safety issues, the safety of the antibiotic-resistance marker gene should be verified through comprehensive evaluation covering the following matters.

- Mechanism of resistance expression, method of use, and associated metabolites

The administration routes (oral, intravenous, etc.) of the antibiotic should be described. The mechanism of antibiotic resistance should be explained. Scientific rationales should be presented to ensure that metabolites associated with resistance expression do not pose any safety issues.

- Information on the other antibiotics (method of use, amount of use, purpose of use, etc.) possibly related to the resistance should be provided.

- The origin of the inserted antibiotic-resistance marker genes should be the same as that of the existing antimicrobial resistance bacteria.

- Scientific rationales should be presented to ensure the absence of any safety issues associated with inactivation of the antibiotic, considering the intake of the gene product (protein) of the antibiotic-resistance marker gene, its degradation during the cooking process and within the digestive tract, and the conditions of use of the antibiotic.

5. Proallergic property of the gene product (protein) (including the gene product of antibiotic-resistance marker genes (e.g. antibiotics metabolizing enzyme))

The safety of the gene product should be confirmed based on the following items (1) to (4). If the safety is not confirmed even based on the items (1) to (4), item (5) in addition to item (1) to (4) shall be evaluated for the confirmation. All the items are not necessarily mandatory when appropriate scientific rationales are provided.

(1) Information on the proallergic property (hereafter including the induction of gluten-sensitive enteropathy) of the donor of the inserted gene (including antibiotic-resistance marker genes) should be provided.

(2) Information on the proallergic property of the gene product (protein) itself should be provided.

(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, (i) to (iii), 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).

(i) Acidic and enzymatic (pepsin) treatment with artificial gastric fluid

(ii) Alkaline and enzymatic (pancreatin) treatment with artificial intestinal fluid

(iii) 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 indicate 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 provided.

(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 (i) to (iv).

(i) If the donor of the inserted gene is proallergic, sera with high titers of donor-specific IgE should be used.

(ii) If 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,

(iii) If the gene product have no sequence homology with any known allergen, but its proallergic property 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,

(iv) If appropriate sera for (i) to (iii) 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 inserted gene has proallergic property, 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.

6. Stability of the gene introduced into the recombinant

- Passage culture should be performed over a sufficient number of generations. The stability of the introduced gene(s), i.e. the structure and the introduced cite of the introduced gene(s) as well as levels of its expression, should be confirmed by Southern and Western blottings or other methods.

- The traits conferred to the microorganism by the introduced genes should be monitored over several generations to confirm the stability.

7. Effect of the expressed gene product on the metabolic pathways

If the gene product is an enzyme, its substrate specificity should be clarified. In principle, the substrate specificity of the enzyme should be high. Supporting rationale shall be provided for ensuring that the substrate specificity has not been changed through the gene insertion event. If the substrate specificity is originally low or has been changed, scientific rationales should be presented to ensure that it does not pose any safety issues.

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 ensure that the alterations do not pose any safety issues.

8. Differences from the host

Based on comparative data between the recombinant and the host etc., it should be clarified whether or not the recombinant is significantly different from the host in terms of non-pathogenicity and production of harmful physiologically active substances. If any significant differences are revealed, scientific rationales should be presented to ensure that the differences do not pose any safety issues.

9. Inactivation

The methods for inactivating or the conditions for sterilizing the recombinant should be described.

10. Handling, storing, and managing of the recombinant

In principle, no difference in handing, storing, and managing should be recognized between on the host and on the recombinant, based on the provided information. If any differences are recognized, scientific rationales should be presented to ensure that the differences do not raise any safety issues. The strains of microorganisms before modification (host) and after modification (recombinant) should be submitted, if requested in the safety assessment

Chapter 3 Safety Assessment of Foods Produced Using Genetically Modified Microorganisms

After completing the safety assessment on the recombinant microorganisms (recombinants) in Chapter 2, the safety assessment of foods produced using genetically modified microorganisms (GMMs) should be conducted product by product, depending on the manufacturing process and final products. The products are classified into either of the two categories; I) GMFMs without living recombinants; II) GMFMs with living recombinants. The points to be considered in the assessment are shown below in each category. The additional guidelines may be developed, if needed, depending on the differences in the manufacturing process and the nature of the products.

In cases where the safety assessment on the recombinant has already been conducted, only the safety assessment in Chapter 3 will be conducted on GMFMs.

II.Safety Assessment on GMFMs Without Living Recombinants

Section 1 Bases for processing as GMFMs without living recombinants

1. Confirm that living recombinants are not contained in the final product

   The proliferation of the recombinant in question should not be observed in the study where the final product is cultured with a suitable culture medium and the optimum temperature for proliferation.

Section 2 Conventional food products comparable with GMFMs in the safety assessment

1. The history of use (i.e. diet culture) or the industrial experience in the production of comparable conventional foods (conventional foods) should be provided.
2. Manufacturing methods (i.e., raw materials, manufacturing process, etc.) of conventional foods should be described.
3. If the conventional foods contain harmful bioactive substances, the kinds and effects as well as contents of the substances should be clarified. Also, the information on proallergic property of the foods in question should be provided.

Section 3 GMFMs

1. Manufacturing methods

   In cases where the manufacturing methods up through the final product are different from those of conventional foods, the differences should be clarified. In such cases, the kinds and amounts of hazardous substances possibly mixed in during the production process should be predictable. Also scientific rationales should be presented to ensure that the substances do not raise any safety issues.

2. Major nutrients

   Changes in the composition of major nutrients (protein, lipid, etc.) should be clarified to assess possible changes in intakes of nutrients in the case where the GMFMs are placed on the market.

3. Safety of substances originated from manufacturing

Compared to conventional food, contents of substances originated from manufacturing should not be significantly changed. In addition, any new substances that are not observed in the conventional foods should not be contained, except for the intentionally produced substances.

Otherwise, scientific rationales should be presented to ensure that the changes in contents of substances and/or the existence of new substances do not pose any safety issues. In particular, far higher levels of metabolites may be produced in foods produced using the recombinant than in conventional foods. The content fluctuations of the substance, which may affect human health, should be the same as those observed in conventional foods. If different, scientific rationales should be presented to ensure that the difference in the content fluctuations does not pose any safety issues. In cases where proallergic substances might be generated anew during the manufacturing process, a safety assessment on the substances should be conducted.

4. Effects on other microorganisms coexisting in the manufacturing process

When the recombinant microorganisms are used under the conditions where other microorganisms coexist in the manufacturing process, the existence of effects on other microorganisms should be described. In addition, scientific rationales should be presented to ensure that the gene transfer to the other microorganisms from the recombinant does not pose any safety issues.

5. Approval and utilization in foods in other countries

Information on the approvals of the recombinant by foreign authorities and its utilization in foods should be provided.

Section 4 Studies required additionally when safety cannot be confirmed based on the sections in Chapter 2 and Sections 2 and Section 3 in Chapter 3, II.

The safety of the food produced using recombinants should be confirmed based on results of appropriate studies 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)

III. Safety Assessment on Products with Living Recombinants

Section 1 Reasons for categorizing in products with living recombinant microorganisms

1. To confirm that living recombinant microorganisms are contained in the final product

The proliferation of recombinant in question should be observed in the study where the final product is cultured with a suitable medium at the optimum temperature for the proliferation. If the existence of living recombinants could not be confirmed, the assessment should be conducted presuming the existence of living recombinants.

Section 2 Conventional food products used for comparators in the safety assessment of GMFMs

1. The history of use (i.e. diet culture) or the industrial experience in the production of comparable conventional foods (conventional foods) should be provided.
2. Manufacturing methods (i.e., raw materials, manufacturing process, etc.) of conventional foods should be described.
3. If the conventional foods contain harmful bioactive substances, the kinds and effects as well as contents of the substances should be clarified. Also, information on any proallergic properties of the foods in question should be provided.

Section 3 GMFMs

1. Manufacturing methods

   In cases where the manufacturing methods up through the final product are different from those of conventional foods, the differences should be clarified. In such cases, the kinds and amounts of hazardous substances possibly contaminated during the production process should be predictable. Moreover, scientific rationales should be presented to ensure that the substances do not pose any safety issues.

2. Major nutrients

   Changes in the composition of major nutrients (protein, lipid, etc.) should be described to assess possible changes in intakes of nutrients in the case where the GMFMs are placed on the market.

3. Safety of substances originated from manufacturing

   Compared to conventional food, contents of substances originated from manufacturing should not be significantly changed. In addition, any new substances that are not observed in the conventional foods should not be contained, except for intentionally produced substances.

   Otherwise, scientific rationales should be presented to ensure that the changes in contents of substances and/or the existence of new substances do not pose any safety issues. In particular, far higher levels of metabolites may be produced in foods produced using the recombinant than in conventional foods. The content fluctuations of the substance, which may affect human health, should be the same as those observed in conventional foods. If different, scientific rationales should be presented to ensure that the difference in the content fluctuations does not pose any safety issues. In cases where proallergic substances might be generated anew during the manufacturing process, the safety assessment on the substances should be conducted.

4. Effects on other microorganisms coexisting in the manufacturing process

   When the recombinant microorganisms are used under conditions where other microorganisms coexist in the manufacturing process, the existence of effects on other microorganisms should be described.

5. Viability and colonization of recombinants in the human digestive tracts

   Scientific rationales should be presented to ensure that no safety issues are identified concerning the viability and colonization of the recombinant in the human digestive tract.

6. Gene transfer

   Scientific rationales should be presented to ensure that no safety issues are identified concerning the gene transfer from the recombinant to other microorganisms, including intestine-inhabiting microorganisms and to epithelial cells of digestive tract.

7. Effects on intestinal flora

Scientific rationales should be presented to ensure that no safety issues are identified concerning the effects of the recombinant on human intestinal flora.

8. Effects on the intestinal system and immune system

Scientific rationales should be presented to ensure that no safety issues are identified concerning the effects on the human intestinal system and immune system.

9. Possibility of infection

Scientific rationales should be presented to ensure that no safety issues are identified concerning the infection of the recombinant to humans and animals.

10. Approval and utilization as food in other countries

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

Section 4 Studies required additionally when safety cannot be confirmed based on the sections in Chapter 2 and Sections 2 and Section 3 in Chapter 3, II.

The safety of the food produced using recombinants should be confirmed based on results of appropriate studies 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)