Standards for Safety Assessments of Food Additives produced Using Genetically Modified Microorganisms

(Food Safety Commission Decision of March 25, 2004)

Chapter 1 General Provisions

No. 1 Background on the establishment of standards for assessment

Based on the Guideline for Safety Assessments of Foods and Food Additives Produced by Recombinant DNA technologies established in 1991 by the Ministry of Health and Welfare (at that time), the first safety assessment of food additives produced by recombinant DNA technology was conducted in 1994, and in 1996, safety assessment of genetically modified foods derived from seed plants was conducted. Since then, confirmation of the safety of a number of genetically modified foods and food additives has been carried out. Moreover, following a revision of the standards for food and food additives under the provisions of Food Sanitation Act, since April 2001 safety assessments of genetically modified foods have been mandatory. In July 2003, Food Safety Commission was newly established, and subsequently, safety assessments of genetically modified foods and food additives, have been conducted by Food Safety Commission at the request of Ministry of Health, Labour and Welfare.

In these Standards, the principles and matters necessary for the assessment of the safety of additives produced using genetically modified microorganisms (hereinafter referred to as "Genetically Modified Additives") to be conducted by the Food Safety Commission, which are considered on the basis of the Ministry of Health, Labour, and Welfare's safety investigation standards, are prescribed as Safety Assessment Standards.

No. 2 Definitions

1 Recombinant DNA technology

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

2 Host

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

3 Vectors

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 Donors

A microorganism, animal, or plant that supplies inserted DNA

7 Expression vector

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

8 Recombinant

A host containing recombinant DNA

9 Gene product

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

10 Genetically modified microorganisms

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

No. 3　Target additives and the purpose of these Standards

The purpose of these Standards is to provide assessment criteria required for assessment of the safety of genetically modified additives.

Genetically modified additives targeted in these Standards are to be within the range of additives which are approved under the Food Sanitation Law. In principle, additives produced using microorganisms that fall under "cases where DNA ultimately introduced to the host through recombinant DNA technology is only DNA from a microorganism belonging to the same taxonomic species as said microorganism" or "cases where living cells that have an equivalent genetic structure to that of the recombinant exist in the natural world" are not included. However, if it is determined that the content and degree of the effects on human health of said additive is not clear, the effects are to be examined as required. Also, if the genetically modified microorganisms (recombinants) used in production remain, there is a need to simultaneously meet the standards of the safety assessment concerning said genetically modified foods (microorganisms) prescribed respectively.

The objective of these Standards is not the examination of matters concerning the environmental, ethical, moral, and socioeconomic aspects of research and development, production and marketing of genetically modified additives.

No. 4 Principles and fundamental approach for safety assessment of genetically modified additives

In respect of genetically modified additives, generally speaking it is appropriate to conduct safety assessments of additive products as end products, different in comparison to those of genetically modified foods in which the recombinant is eaten as is. For this reason, it is important that genetically modified additives contain no new harmful substances that come about due to the application of recombinant DNA technology.

Accordingly, if additives of microbial origin are produced using genetically modified microorganisms (recombinants), it is reasonable to conduct safety assessments focusing on the recombinant-derived components that are newly added to conventional additives.

However, in cases where animal enzymes are produced using genetically modified microorganisms, it is necessary that safety assessments are conducted concerning the recombinant-derived components, comparing non-active ingredients of foreign substances derived from each of both the recombinant and the host with history of safe use, in addition to the active ingredients from each of conventional additives and genetically modified additives.

In any case, it is necessary that safety assessment of the recombinant (genetically modified microorganism) used in production of said additive is conducted by comparing the recombinant used in the production of said additives and the existing host. In this assessment, it is necessary that not only both qualitative and quantitative changes in active ingredients that are produced intentionally, but also both qualitative and quantitative changes in non-active ingredients of foreign substances that may be mixed unintentionally, are taken into account.

At the same time, there are an extremely wide variety of additives in terms of properties, intended uses, manufacturing methods, and other points. Moreover, macromolecular additives, especially food enzymes are often denatured and deactivated during the manufacturing process of food and are eventually removed from the food. For this reason, the safety assessments of genetically modified additives shall be conducted on a case-by-case basis, taking into account the degree of refinement of said additives, patterns of usage, residue in food, and other matters as necessary.

In accordance with the principles described above, safety assessments shall be conducted based on the following basic approach:

1　In principle, safety assessments of genetically modified additives are deemed to be feasible only in cases where recombinants derived from nonpathogenic hosts with a

history of use in the production of additives or a history of use as foods are used, and also when comparison with additives approved by Food Sanitation Act is possible.

2 The assessment of the safety of genetically modified additives is conducted in order to clarify the impossibility that any of the changes in the characteristics expected to be conferred to the host through recombinant DNA technology will have an unexpected harmful effect on human health.

The characteristics that should be considered when conducting such safety assessment of recombinants include antinutrient content, endogenous toxins, allergenic substances, physiologically active substances, and secondary effects arising from changes in metabolic pathways in the recombinants attributable to the gene introduction.

3 As described above in the principles, with regard to genetically modified additives, the recombinant is not eaten as is, its method of manufacture and methods and patterns of usage are different from that of genetically modified foods, which themselves are eaten, therefore, the points to be emphasized in safety assessment are different. That is to say, it is reasonable to conduct safety assessments for each product on a case-by-case basis, taking into consideration the degree of refinement of genetically modified additives, their patterns of usage, persistence in food including foreign substances and other non-active ingredients that are likely to be unintentionally mixed in, and other matters as required.

For example, in cases where metabolic products such as riboflavin are the active ingredient of an additive and the gene products themselves are not active ingredients, even though recombinants are used in the production of the additive, it is important to clarify that new components derived from the recombinant besides the active ingredient do not remain in the additive or do not pose any safety risk.

Also, in cases where new proteins besides the active ingredient are produced by the recombinant and are not ultimately removed from the genetically modified additives, safety assessments must be conducted on said protein for adverse effects such as toxicity and allergenicity.

Furthermore, in cases where a genetically modified additive is classifiable as a food enzyme, assessments must be conducted for adverse effects such as toxicity and allergenicity as needed when said genetically modified additive involves amino acid substitutions.

4 Tests conducted for safety assessments should follow scientifically reliable concepts and principles and, when needed, should be planned and implemented following GLP. Source data should be submitted as required. Data and information that are required for safety assessments include experimental data prepared by

developers and researchers, previously published scientific articles, and information from third parties. It is important that these data are obtained using scientifically reliable methods and analyzed using appropriate statistical techniques. Also, the lower limit of quantitation of the analytical methods should be shown wherever possible.

5 At present, kanamycin resistance genes used as antibiotic resistance markers have been appropriately assessed for safety and present no immediate safety concerns. For the future development of genetically modified microorganisms used in the production of genetically modified additives, if transformation techniques on which thorough safety assessments are conducted and that do not use antibiotic resistance marker genes become readily available, the use of those techniques should be considered.

6 Since recombinant DNA technology is advancing on a daily basis, these standards for safety assessment will need to be reconsidered as required needed in accordance with the advancement of said technology.

Chapter 2 Standards for safety assessments of genetically modified additives

No.1 Properties of the additives and hosts to be compared in safety assessments, and the differences between the additives and genetically modified additives, as well as those between the hosts and recombinants

It is necessary to describe the outline of the matters stated in the following (1 to 6) and thereby to show that an additive approved by the Food Sanitation Law exists as a comparable additive as required in the safety assessment of genetically modified additives, that properties of the host from which the recombinant used for production of said additive is derived are clarified, and that the differences such as differences between genetically modified additives and conventional additives and differences between recombinants and hosts are clear.

1 Information concerning conventional additives including properties and intended uses

(1) Name, origin, and active ingredient

(2) Method of manufacture

(3) Intended uses and patterns of usage

(4) Amount of intake

2 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 introduction of inserted DNA

3 Information on the host concerning the history of use in production of additives and the history of use as food

4 Information on the host concerning its structural components

If any harmful physiologically active substance or antinutrient (a substance that inhibits the digestion and absorption of nutrients) is included in the host, an overview of the type and amount of such substances

5 Information on genetically modified additives concerning their properties and intended uses

(1) Product name and active ingredient

(2) Method of manufacture

(3) Intended uses and patterns of usage

(4) Properties of active ingredients and comparison to conventional additives

6 Differences between the genetically modified additives and conventional additives, and those between the recombinant and host or others that need to be considered in safety assessments

If it is determined that there is a conventional additive and host that can be used in a comparison with said genetically modified additives and recombinant, the assessment shall be conducted based on the items listed in each matter under No. 2, making said comparisons.

No. 2 Matters related to hosts

1 Matters related to the taxonomic position (species name (scientific name), strain name, etc.) and others

The scientific name, strain name, etc. of the host should be specified, and also, a history of safe use in additive production, a history of safe use in food (diet culture), or experience of industrial use should be provided.

2 Matters related to pathogenicity and production of harmful physiologically active substances

The host should be nonpathogenic. In addition, if the host produces harmful physiologically active substances, the type, effect and amount of such substance should be provided. Information on allergenicity of the host should be provided as required.

3 Matters related to parasitic and fixing properties

Whether or not the host parasitizes or fixes itself in humans or any other organism should be clarified. If the host parasitizes or fixes itself in humans or any other organism, whether or not the host has a harmful effect on humans or any other organism should be clarified.

4 Matters related to not being contaminated by foreign pathogenic factors (such as viruses)

The host used in the development of said recombinant not being contaminated by foreign pathogenic factors (such as viruses).

5 Matters related to pathogenicity and production of harmful physiologically active substances of the host's closely related strain

If there is any pathogenic strain or any strain that may produce harmful physiologically active substances in the strains closely related to the host, whether such pathogenicity or production of such substances exist in said microorganism used for the production of genetically modified additives should be clarified.

In addition, if the production of harmful physiologically active substances is observed, the rationale for determining that there are no safety concerns in production using said microorganisms should be provided.

No. 3 Matters related to vectors

1 Matters related to names and origins

The name and origin of the plasmid or other vectors used for gene transfers should be specified. In addition, the vector should not be known to be harmful to humans.

2 Matters related to properties

(1) Matters on the number of DNA bases and its sequence

The number of DNA bases and its sequence should be identified. In addition, if the sequence of bases is disclosed to the public, the public database identification number should be specified.

(2) Matters related to a restriction map with a restriction enzyme

The restriction map of the vector should be identified. In such a case, the name of restriction enzymes used, as well as the number and size of fragments should be specified.

(3) Matters related to not containing any base sequences which are known to be harmful.

The vector should contain no base sequences which are known to be harmful.

(4) Matters related to drug resistance

If a drug resistance gene is contained in the vector, the properties of the gene should be clarified.

(5) Matters related to the transmissibility

In principle, the vector should show no transmissibility (i.e. the potential of the vector to autonomously migrate from the host microorganism to other strains [i.e.

horizontal transmission]). If the vector is transmissible, the range of target organisms should be clarified.

(6) Matters related to host dependency

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

No. 4 Matters related to inserted DNA, gene products, and expression vector construction

1 Matters related to the donor of the inserted DNA

(1) Matters related to name, origin and classification

Name, origin and classification should be specified.

(2) Matters related to safety

· The donor of the inserted DNA should not be known to have any pathogenicity to humans or to produce any toxins. In addition, if any pathogenic strain is known within the donor species, as in the case of Echerichia Coli, it should be clarified that the donor has been derived from a nonpathogenic strain.

· If the donor has been reported to be pathogenic or to produce a toxin, it should be clarified that the inserted DNA itself does not produce any toxin, and that the protein(s) derived from the inserted DNA is nonpathogenic.

· It should be clarified whether the donor of the inserted gene has a history of safe consumption.

2 Matters related to properties of the inserted DNA or genes (including antibiotic resistance marker genes) and their gene products

(1) Matters related to methods for cloning or synthesizing inserted genes

Methods used for cloning or synthesizing inserted genes should be described.

(2) Matters related to number of bases and sequence, and a restriction map with a restriction enzyme

The number of bases and sequence of DNA fragments to be introduced into the host should be specified. The restriction map should also be provided with the names of the restriction enzyme, and the number and sizes of DNA fragments.

(3) Matters related to the functions of inserted genes

The functions of inserted genes and the properties and functions of its products (RNA and protein) should be clarified. The rationale for determining that the protein has no adverse effects should be presented.

In particular, in cases where said gene products (protein) involve amino acid substitutions and will be used as is as a food enzyme, the rational for determining that

there are no safety issues associated with toxicity, allergenicity, or other adverse actions of said gene products (protein) should be presented, having given due consideration as needed to usage patterns in the food production process and to the estimated amount remaining in the end food product.

3 Matters related to areas involved in the expression of inserted genes and antibiotic resistance marker genes

(1) Matters related to promoters

The origin and properties of promoters used should be described.

(2) Matters related to terminators

The origin and properties of terminators used should be described.

(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 Matters related to methods for incorporating inserted DNA to vectors

The methods for incorporating inserted DNA to vectors should be provided, specifically the following:

· 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 gene fragments.

· 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 Matters related to constructed expression vectors

(1) Matters related to the number of bases and sequence, and a restriction map with a restriction enzyme

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

(2) In principle, the expression 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 within the recombinant, the rationale for determining the safety of the gene and its product should be presented.

(3) The intended insertion area in the expression vector should be clearly indicated in the method used to introduce genes into the host.

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

6 Matters related to methods for introducing DNA into the host

The method for introducing inserted DNA into the host, including the plasmid or the DNA construct which are used in expression, should be provided. Specifically,

· the method for introducing 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) and

· the selection method (the method used for selecting the host to which the DNA is introduced) should be described.

7 Matters related to safety of the 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 it is unclear that the marker gene and its product are, during the process of additive production, 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, including transformation in the recombinant:

(1) Matters related to attributes of the gene and its gene product

· Structure and function

The base sequence of the inserted antibiotic resistance marker gene should be identified and the gene should not contain any other harmful base sequences.

The function of the gene product (protein) should be explained. If needed, substrate specificity should be identified.

The primary structure of the gene product should be compared to that of known allergens and no structural homology should exist between them.

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

The administration routes (oral, intravenous, etc.) of the antibiotic should be specified. The mechanism of antibiotic resistance should be explained. The rationale for determining the safety of metabolites associated with resistance expression should be provided.

· Identification and quantitative methods

There should be methods for identifying and quantifying the gene product (protein) derived from the antibiotic resistance gene, and the expression level should be determined.

· Sensitivity of the gene product (protein) to various treatments

Data on any alterations in the molecular weight, enzymatic activity, and immunoreactivity of the gene product (protein) by acidic and enzymatic treatment with artificial gastric fluid, alkaline and enzymatic treatment with artificial intestinal fluid,

and heat and other physical treatments should be provided. The rationale for determining that these alterations pose no safety issues should be presented.

· Allergenicity

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

(2) Matters related to intake of the gene and its gene products

· The conditions of use (e.g. methods, amounts, and purpose of use) of the antibiotics to which the gene conferring resistance should be clarified.

· The origin of the inserted antibiotic resistance marker gene should be similar to normally-present antibiotic resistant bacteria.

· The rationale for determining that there is no problem accompanied with the inactivation of the antibiotic should be presented, considering the intake of the gene product (protein) of the antibiotic resistance marker gene, degradation during the cooking process and within the digestive tract, and the conditions of use of the antibiotic.

No. 5 Matters related to recombinants

1 Matters related to differences from the host

Based on comparative data between the recombinant and the host, differences in non-pathogenicity and non-production of harmful, physiologically active substances should be clarified, and there should be no safety issues.

2 Matters related to gene insertion

(1) Matters related to a restriction map with a restriction enzyme

A restriction map of the DNA fragment introduced into the host should be provided. In such a case, the names of the restriction enzymes, the number and sizes of DNA fragments, and patterns observed in Southern blot analysis should be provided.

(2) Matters related to the presence or absence of open reading frames and the possibility of their transcription and expression

· In principle, the rationale for determining that the DNA inserted into the host DNA contains no open reading frame that expresses an unintended protein should be presented. The impossibility of DNA insertion leading to expression of an unintended protein should be verified using methods such as northern blotting and RT-PCR.

· If any gene that can express an unintended protein is contained, the rationale for determining that the gene and its product protein do not pose any safety issues, including allergenicity, should be presented.

No.6 Raw materials besides the recombinant and production equipment

1　The raw materials and the production equipment used for the additive should have usage records as those of additives.

2　Information on the safety of the raw materials and the production equipment for the additive should be documented.

(If the conditions described 1 and 2 above are not confirmed, the safety of the raw materials and production equipment when used for food or additives should be clarified.)

No. 7 Matters related to genetically modified additives

1　Matters related to approval and food usage in foreign countries

Information on the status of approval in foreign countries should be provided. Information on whether or not the said item is being used as an additive should also be provided.

2　Matters related to recombinant residues

The verification of whether residue of a recombinant exists or not should be conducted through appropriate tests such as dot-blot hybridization using samples from the most suitable process.

3　Matters related to safety of non-active ingredients derived from production

The content of non-active ingredients derived from production should not be significantly increased compared to that of the conventional additive. Also, there should be no non-active ingredients that are not present in the conventional additive. In all other cases, the rationale for determining that non-active ingredients pose no safety issues should be presented.

4　Matters related to the method of purification and its effectiveness

The method of purification and its effectiveness should be clarified. The kind and quantity of harmful substances that could potentially become mixed in during the production process should be possible to be estimated, and the rationale for determining that said substances pose no safety concerns should be presented.

5　Changes in ordinary components which suggest toxicity due to changes in content

For an ordinary component which suggests toxicity due to changes in its content, changes in their concentration should be the same as those of the conventional additive. Even if there is a change, the rationale for determining that this poses no safety concern should be provided.

No.8 Additional studies required when safety cannot be confirmed based on No. 2 to 7 above

Safety as an additive should be confirmed based on results of those considered necessary among the following:

(1) Acute toxicity study

(2) Subacute toxicity study

(3) Chronic toxicity study

(4) Reproductive toxicity study

(5) Mutagenicity study

(6) Carcinogenicity study

(7) Other necessary studies (e.g. intestinal toxicity study, immunotoxicity study, neurotoxicity study, nutrition study)