THAI AGRICULTURAL STANDARD

TAS 9013-2006

GUIDELINE FOR THE CONDUCT OF FOOD SAFETY ASSESSMENT OF FOODS PRODUCED USING RECOMBINANT-DNA MICROORGANISMS

National Bureau of Agricultural Commodity and Food Standards
Ministry of Agriculture and Cooperatives

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The production of foods using recombinant-DNA microorganisms is becoming important in international trade. Codex Alimentarius Commission, Joint FAO/Who Food Standards Programme, had developed the Guideline for the Conduct of Food Safety Assessment of Foods Produced Using Recombinant-DNA Microorganisms as a guideline to be implemented by countries.

According to the international acceptance of the Codex safety assessment guideline, it is appropriate to establish a national standard which is based on the Codex guideline.

The establishment of this standard is based on the following document:

FAO/WHO. 2004. Guideline for the Conduct of Food Safety Assessment of Foods Produced Using Recombinant-DNA Microorganisms, pp. 27-46. In Codex Alimentarius Commission: Foods Derived from Biotechnology. Joint FAO/WHO Food Standards Programme, FAO, Rome.

***Remark*:**

The standard title has been revised from “Thai Agricultural Commodity and Food Standard (TACFS)” to “Thai Agricultural Standard (TAS)” in accordance with the enforcement of the Agricultural Standards Act B.E. 2551 (2008).

NOTIFICATION OF THE NATIONAL COMMITTEE ON AGRICULTURAL COMMODITY AND FOOD STANDARDS
SUBJECT: THAI AGRICULTURAL COMMODITY AND FOOD STANDARD: GUIDELINE FOR THE CONDUCT OF FOOD SAFETY ASSESSMENT OF FOODS PRODUCED USING RECOMBINANT-DNA MICROORGANISMS
B.E.2549 (2006)

The resolution of the 1/2549 session of the National Committee on Agricultural Commodity and Food Standards dated 8 June B.E. 2549 (2006) endorsed the Thai Agricultural Commodity and Food Standard entitled Guideline for the Conduct of Food Safety Assessment of Foods Produced Using Recombinant-DNA Microorganisms. This standard would be of benefits for quality improvement, facilitating trade and protecting consumers.

By virtue of the Cabinet Resolution on Appointment and Authorization of the National Committee on Agricultural Commodity and Food Standards dated 19 November B.E.2545 (2002), the Notification on Thai Agricultural Commodity and Food Standard entitled Guideline for the Conduct of Food Safety Assessment of Foods Produced Using Recombinant-DNA Microorganisms is hereby issued as voluntary standard, the details of which are attached herewith.

Notified on 19 July B.E.2549 (2006)

Khunying Sudarat Keyuraphan

Minister of Agriculture and Cooperatives
Chairperson of the National Committee on Agricultural Commodity and Food Standards

THAI AGRICULTURAL STANDARD

GUIDELINE FOR THE CONDUCT OF FOOD SAFETY ASSESSMENT OF FOODS PRODUCED USING RECOMBINANT-DNA MICROORGANISMS

1 SCOPE

This Thai Agricultural Standard on Guideline for the Conduct of Food Safety Assessment of Foods Produced Using Recombinant-DNA Microorganisms should be used in conjunction with the Thai Agricultural Standard on Principles for the Risk Analysis of Foods Derived from Modern Biotechnology (TAS 9010) and Thai Agricultural Standard on Assessment of Possible Allergenicity (TAS 9011) This Guideline supports the Principles for the Risk Analysis of Foods Derived from Modern Biotechnology and addresses safety and nutritional aspects of foods produced through the actions of recombinant-DNA microorganisms. [1/] The recombinant-DNA microorganisms that are used to produce these foods are typically derived using the techniques of modern biotechnology from strains that have a history of safe, purposeful use in food production. However, in instances where the recipient strains do not have a history of safe use their safety will have to be established. [2/] Such food and food ingredients may contain viable or non-viable recombinant-DNA microorganisms or may be produced by fermentation using recombinant-DNA microorganisms from which the recombinant-DNA microorganisms may have been removed.

Recognizing that the following issues may have to be addressed by other bodies or other instruments, this document does not address:

(1) safety of microorganisms used in agriculture (for plant protection, biofertilizers, in animal feed or food derived from animals fed the feed etc.);

(2) risks related to environmental releases of recombinant-DNA microorganisms used in food production;

(3) safety of substances produced by microorganisms that are used as additives or processing aids, including enzymes for use in food production[3/];

[1/] The Joint FAO/WHO Expert Committee on Food Additives (JECFA) is revising guidelines for General Specifications and Considerations for Enzyme Preparations used in food processing. These guidelines have been used to evaluate enzyme preparations derived from genetically modified microorganisms.

[2/] The Joint FAO/WHO Expert Committee on Food Additives (JECFA) is revising guidelines for General Specifications and Considerations for Enzyme Preparations used in food processing. These guidelines have been used to evaluate enzyme preparations derived from genetically modified microorganisms.

[3/] The Joint FAO/WHO Expert Committee on Food Additives (JECFA) is revising guidelines for General Specifications and Considerations for Enzyme Preparations used in food processing. These guidelines have been used to evaluate enzyme preparations derived from genetically modified microorganisms.

(4) specific purported health benefits or probiotic effects that may be attributed to the use of microorganisms in food; or

(5) issues relating to the safety of food production workers handling recombinant-DNA microorganisms.

A variety of microorganisms used in food production have a long history of safe use that predates scientific assessment. Few microorganisms have been assessed scientifically in a manner that would fully characterize all potential risks associated with the food they are used to produce, including, in some instances, the consumption of viable microorganisms. Furthermore, the Codex principles of risk analysis, particularly those for risk assessment, are primarily intended to apply to discrete chemical entities such as food additives and pesticide residues, or specific chemical or microbial contaminants that have identifiable hazards and risks; they were not originally intended to apply to intentional uses of microorganisms in food processing or in the foods transformed by microbial fermentations. The safety assessments that have been conducted have focused primarily on the absence of properties associated with pathogenicity in these microorganisms and the absence of reports of adverse events attributed to ingestion of these microorganisms, rather than evaluating the results of prescribed studies. Further, many foods contain substances that would be considered harmful if subjected to conventional approaches to safety testing. Thus, a more focused approach is required where the safety of a whole food is being considered.

Information considered in developing this approach includes:

(1) uses of living microorganisms in food production;

(2) consideration of the types of genetic modifications likely to have been made in these organisms;

(3) the types of methodologies available for performing a safety assessment; and

(4) issues specific to the use of the recombinant-DNA microorganism in food production, including its genetic stability, potential for gene transfer, colonization of the gastrointestinal tract and persistence[4/]therein,

This approach is based on the principle that the safety of foods produced using recombinant-DNA microorganisms is assessed relative to the conventional counterparts that have a history of safe use, not only for the food produced using a recombinant-DNA microorganism, but also for the microorganism itself. This approach takes both intended and unintended effects into account. Rather than trying to identify every hazard associated with a particular food or the microorganism, the intention is to identify new or altered hazards relative to the conventional counterpart.

This safety assessment approach falls within the risk assessment framework as discussed in Section 4 of TAS 9010. If a new or altered hazard, nutritional or other food safety concern is identified by the safety assessment, the risk associated with it would first be assessed to determine its relevance to human health. Following the safety assessment and, if necessary,

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[4/] The Joint FAO/WHO Expert Committee on Food Additives (JECFA) is revising guidelines for General Specifications and Considerations for Enzyme Preparations used in food processing. These guidelines have been used to evaluate enzyme preparations derived from genetically modified microorganisms.

further risk assessment, the food or component of food, such as a microorganism used in production, would be subjected to risk management considerations in accordance with TAS 9010 before it is considered for commercial distribution.

Risk management measures such as post-market monitoring of consumer health effects may assist the risk assessment process. These are discussed in TAS 9010.

The Guideline describes approaches recommended for making safety assessments of foods produced using recombinant-DNA microorganisms, using comparison to a conventional counterpart. The safety assessment will focus on the safety of the recombinant-DNA microorganisms used in food production, and, where appropriate, on metabolites produced by the action of recombinant-DNA microorganisms on food. The Guideline identifies the data and information that are generally applicable to making such assessments. When conducting a comparison of a recombinant-DNA microorganism or a food produced using recombinant-DNA microorganism with their respective conventional counterparts, any identified differences should be taken into account, whether they are the result of intended or unintended effects. Due consideration should be given to the interactions of the recombinant-DNA microorganism with the food matrix or the microflora and to the safety of any newly-expressed protein(s) and secondary metabolic products. While this Guideline is designed for foods produced using recombinant-DNA microorganisms or their components, the approach described could, in general, be applied to foods produced using microorganisms that have been altered by other techniques.

2 DEFINITIONS

For the purpose of this standard:

2.1 **Recombinant-DNA Microorganism** means bacteria, yeasts or filamentous fungi in which the genetic material has been changed through *in vitro* nucleic acid techniques including recombinant deoxyribonucleic acid (DNA) and direct injection of nucleic acid into cells or organelles.

2.2 **Conventional counterpart**[5/] means:

• a microorganism/strain with a known history of safe use in producing and/or processing the food and related to the recombinant-DNA strain. The microorganism may be viable in the food or may be removed in processing or rendered non-viable during processing; or

• food produced using the traditional food production microorganisms for which there is experience of establishing safety based on common use in food production.

3 INTRODUCTION TO FOOD SAFETY ASSESSMENT

[5/] The Joint FAO/WHO Expert Committee on Food Additives (JECFA) is revising guidelines for General Specifications and Considerations for Enzyme Preparations used in food processing. These guidelines have been used to evaluate enzyme preparations derived from genetically modified microorganisms.

Most foods produced as a result of the purposeful growth of microorganisms have their origins in antiquity, and have been deemed safe long before the emergence of scientific methods for assessing safety. Microorganisms possess properties, such as fast growth rates, that enable genetic modifications, whether employing conventional techniques or modern biotechnology, to be implemented in short time frames. Microorganisms used in food production derived using conventional genetic techniques have not customarily been systematically subjected to extensive chemical, toxicological, epidemiological, or medical evaluations prior to marketing. Instead microbiologists, mycologists, and food technologists have evaluated new strains of bacteria, yeasts and filamentous fungi for phenotypic characteristics that are useful in relation to food production.

Safety assessments of recombinant-DNA microorganisms should document the use of related microorganisms in foods, the absence of properties known to be characteristic of pathogens in the recombinant-DNA microorganisms or the recipient strains used for constructing the recombinant-DNA microorganisms, and known adverse events involving the recipient or related organisms. In addition, when a recombinant DNA microorganism directly affects or remains in the food, any effects on the safety of the food should be examined.

The use of animal models for assessing toxicological effects is a major element in the risk assessment of many compounds, such as pesticides. In most cases, however, the substance to be tested is well characterized, of known purity, of no particular nutritional value, and human exposure to it is generally low. It is therefore relatively straightforward to feed such compounds to animals at a range of doses some several orders of magnitude greater than the expected human exposure levels, in order to identify any potential adverse health effects of importance to humans. In this way, it is possible, in most cases, to estimate levels of exposure at which adverse effects are not observed and to set safe intake levels by the application of appropriate safety factors.

Animal studies cannot readily be applied to testing the risks associated with whole foods, which are complex mixtures of compounds, and often characterized by a wide variation in composition and nutritional value. Due to their bulk and effect on satiety, they can usually only be fed to animals at low multiples of the amounts that might be present in the human diet. In addition, a key factor to consider in conducting animal studies on foods is the nutritional value and balance of the diets used, in order to avoid the induction of adverse effects that are not related directly to the material itself. Detecting any potential adverse effects and relating these conclusively to an individual characteristic of the food can therefore be extremely difficult. If the characterization of the food indicates that the available data are insufficient for a thorough safety assessment, properly designed animal studies could be requested on the whole food. Another consideration in deciding the need for animal studies is whether it is appropriate to subject experimental animals to such a study if it is unlikely to give rise to meaningful information.

Animal studies typically employed in toxicological evaluations also cannot be readily applied to testing potential risks associated with ingestion of microorganisms used for food production. Microorganisms are living entities, containing complex structures composed of many biochemicals, and therefore are not comparable to pure compounds. In some processed foods, they can survive processing and ingestion and can compete and, in some cases, be retained in the intestinal environment for significant periods of time. Appropriate animal studies should be used to evaluate the safety of recombinant-DNA microorganisms where the donor, or the gene or gene product do not have a history of safe use in food, taking into

account available information regarding the donor and the characterization of the modified genetic material and the gene product. Further, appropriately designed studies in animals may be used to assess the nutritional value of the food or the bioavailability of the newly expressed substance in the food.

Due to the difficulties of applying traditional toxicological testing and risk assessment procedures to whole foods, a more focused approach is required for the safety assessment of foods produced using recombinant-DNA microorganisms. This has been addressed by the development of a multidisciplinary approach for assessing safety that takes into account the intended effect, the nature of the modification and detectable unintended changes that may occur in the microorganism or in its action on the food, using the concept of substantial equivalence.

While the focus of a safety assessment will be on the recombinant-DNA microorganism, additional information on its interaction with the food matrix should be taken into consideration when applying the concept of substantial equivalence, which is a key step in the safety assessment process. However, the concept of substantial equivalence is not a safety assessment in itself. Rather it represents the starting point that is used to structure the safety assessment of both a recombinant-DNA microorganism relative to its conventional counterpart and the food produced using recombinant-DNA microorganism relative to its conventional counterpart. This concept is used to identify for evaluation similarities and differences between a recombinant-DNA microorganisms used in food processing as well as the food produced using the recombinant-DNA microorganisms and their respective conventional counterparts as defined in paragraph 9. It aids in the identification of potential safety and nutritional issues and is considered the most appropriate strategy to date for safety assessment of foods produced using recombinant-DNA microorganisms. The safety assessment carried out in this way does not imply absolute safety of the new product; rather, it focuses on assessing the safety of any identified differences so that the safety of the recombinant-DNA microorganism and the food produced using recombinant-DNA microorganism can be considered relative to their respective conventional counterparts.

3.1 UNINTENDED EFFECTS

In achieving the objective of conferring a specific target trait (intended effect) to a microorganism by the addition, substitution, removal, or rearrangement of defined DNA sequences, including those used for the purpose of DNA transfer or maintenance in the recipient organism, additional traits could, in some cases, be acquired or existing traits could be lost or modified. The potential for occurrence of unintended effects is not restricted to the use of *in vitro* nucleic acid techniques. Rather, it is an inherent and general phenomenon that can also occur in the development of strains using traditional genetic techniques and procedures, or from exposure of microorganisms to intentional or unintended selective pressures. Unintended effects may be deleterious, beneficial, or neutral with respect to competition with other microorganisms, ecological fitness of the microorganism, the microorganism’s effects on humans after ingestion, or the safety of foods produced using the microorganism. Unintended effects in recombinant-DNA microorganisms may also arise through intentional modification of DNA sequences or they may arise through recombination or other natural events in the recombinant-DNA microorganism. Safety assessment should include data and information to reduce the possibility that a food derived from a recombinant-DNA microorganism would have an unexpected, adverse effect on human health.

Unintended effects can result from the insertion of DNA sequences new to a microorganism into the microbial genome; they may be compared with those observed following the activity of naturally occurring transposable genetic elements. Insertion of DNA may lead to changes in expression of genes in the genome of the recipient. The insertion of DNA from heterologous sources into a gene may also result in the synthesis of a chimeric protein, also referred to as a fusion protein. In addition genetic instability and its consequences need to be considered.

Unintended effects may also result in the formation of new or changed patterns of metabolites. For example, the expression of enzymes at high levels or the expression of an enzyme new to the organism may give rise to secondary biochemical effects, changes in the regulation of metabolic pathways, or altered levels of metabolites.

Unintended effects due to genetic modification may be subdivided into two groups: those that could be predicted and those that are “unexpected.” Many unintended effects are largely predictable based on knowledge of the added trait, its metabolic consequences or of the site of insertion. Due to the expanding knowledge of microbial genomes and physiology, and the increased specificity in function of genetic materials introduced through recombinant-DNA techniques compared with other forms of genetic manipulation, it may become easier to predict unintended effects of a particular modification. Molecular biological and biochemical techniques can also be used to analyse changes that occur at the level of transcription and translation that could lead to unintended effects.

The safety assessment of foods produced using recombinant-DNA microorganisms involves methods to identify and detect such unintended effects and procedures to evaluate their biological relevance and potential impact on food safety. A variety of data and information is necessary to assess unintended effects, because no individual test can detect all possible unintended effects or identify, with certainty, those relevant to human health. These data and information, when considered in total, should provide assurance that the food is unlikely to have an adverse effect on human health. The assessment of unintended effects takes into account the biochemical, and physiological characteristics of the microorganism that are typically selected for improving strains for commercial food or beverage uses. These determinations provide a first screen for microorganisms that exhibit unintended traits. Recombinant-DNA microorganisms that pass this screen are subjected to safety assessment as described in Section 4.

3.2 FRAMEWORK OF FOOD SAFETY ASSESSMENT

The safety assessment of a food produced using a recombinant-DNA microorganism is based on determining the safety of using the microorganism, which follows a stepwise process of addressing relevant factors that include:

(1) Description of the recombinant-DNA microorganism;

(2) Description of the recipient microorganism and its use in food production;

(3) Description of the donor organism(s);

(4) Description of the genetic modification(s) including vector and construct;

(5) Characterization of the genetic modification(s);

(6) Safety assessment:

(6.1) expressed substances: assessment of potential toxicity and other traits related to pathogenicity;

(6.2) compositional analyses of key components;

(6.3) evaluation of metabolites;

(6.4) effects of food processing;

(6.5) assessment of immunological effects;

(6.6) assessment of viability and residence of microorganisms in the human gastrointestinal tract;

(6.7) antibiotic resistance and gene transfer; and

(6.8) nutritional modification.

In certain cases, the characteristics of the microorganisms and/or the foods produced/processed using these microorganisms may necessitate generation of additional data and information to address issues that are unique to the microorganisms and/or food products under review.

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

The goal of each safety assessment is to provide assurance, in the light of the best available scientific knowledge, that the food will not cause harm when prepared or consumed according to its intended use, nor should the organism itself cause harm when viable organisms remain in the food. Safety assessments should address the health aspects for the whole population, including immuno-compromised individuals, infants, and the elderly. The expected endpoint of such an assessment will be a conclusion regarding whether the new food and/or microorganisms are as safe as the conventional counterparts taking into account dietary impact of any changes in nutritional content or value. Where the microorganism is likely to be viable upon ingestion, its safety should be compared to a conventional counterpart taking into account residence of the recombinant-DNA microorganism in the gastrointestinal tract, and where appropriate, interactions between it and the gastrointestinal flora of mammals (especially humans) and impacts of the recombinant-DNA microorganism on the immune system. In essence, the outcome of the safety assessment process is to define the product under consideration in such a way as to enable risk managers to determine whether any measures are needed to protect the health of consumers and if so to make well-informed and appropriate decisions in this regard.

4 GENERAL CONSIDERATIONS

4.1 DESCRIPTION OF THE RECOMBINANT-DNA MICROORGANISM

A description of the bacterial, yeast, or fungal strain and the food being presented for safety assessment should be provided. This description should be sufficient to aid in understanding the nature of the organism or food produced using the organism being submitted for safety assessment. Recombinant-DNA microorganisms used in food production or contained in food should be conserved as stock cultures with appropriate identification using molecular

methods, and preferably, in established culture collections. This may facilitate the review of the original safety assessment. Such stock cultures should be made available to regulatory authorities upon request.

4.2 DESCRIPTION OF THE RECIPIENT MICROORGANISM AND ITS USE IN FOOD PRODUCTION

A comprehensive description of the recipient microorganism or microorganism subjected to the modification should be provided. Recipient microorganisms should have a history of safe use in food production or safe consumption in foods. Organisms that produce toxins, antibiotics or other substances that should not be present in food, or that bear genetic elements that could lead to genetic instability, antibiotic resistance or that are likely to contain genes conferring functions associated with pathogenicity should not be considered for use as recipients. The necessary data and information should include, but need not be restricted to:

(1) identity: scientific name, common name or other name(s) used to reference the microorganism, strain designation, information about the strain and its source, or accession numbers or other information from a recognized culture repository from which the organism or its antecedents may be obtained, if applicable, information supporting its taxonomical assignment;

(2) history of use and cultivation, known information about strain development (including isolation of mutations or antecedent strains used in strain construction); in particular, identifying traits that may adversely impact human health;

(3) information on the recipient microorganism’s genotype and phenotype relevant to its safety, including any known toxins, antibiotics, antibiotic resistance factors or other factors related to pathogenicity, or immunological impact, and information about the genetic stability of the microorganism;

(4) history of safe use in food production or safe consumption in food; and

(5) information on the relevant production parameters used to culture the recipient microorganism.

Relevant phenotypic and genotypic information should be provided not only for the recipient microorganism, but also for related species and for any extra-chromosomal genetic elements that contribute to the functions of the recipient strain, particularly if the related species are used in foods or involved in pathogenic effects in humans or other animals. Information on the genetic stability of the recipient microorganism should be considered including, as appropriate, the presence of mobile DNA elements, i.e. insertion sequences, transposons, plasmids, and prophages.

The history of use may include information on how the recipient microorganism is typically grown, transported and stored, quality assurance measures typically employed, including those to verify strain identity and production specifications for microorganisms and foods, and whether these organisms remain viable in the processed food or are removed or rendered non-viable as a consequence of processing.

4.3 DESCRIPTION OF THE DONOR ORGANISM(S)

Information should be provided on the donor organism(s) and any intermediate organisms, when applicable, and, when relevant, related organisms. It is particularly important to determine if the donor or intermediate organism(s) or other closely related species naturally exhibit characteristics of pathogenicity or toxin production, or have other traits that affect human health. The description of the donor or intermediate organism(s) should include:

(1) identity: scientific name, common name or other name(s) used to reference the organism, strain designation, information about the strain and its source, or accession numbers or other information from a recognized culture repository from which the organism or its antecedents may be obtained, if applicable, and information supporting its taxonomic assignment;

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

(3) information on the organism's genotype and phenotype relevant to its safety including any known toxins, antibiotics, antibiotic resistance factors or other factors related to pathogenicity, or immunological impact; and

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

4.4 DESCRIPTION OF THE GENETIC MODIFICATION(S) INCLUDING VECTOR AND CONSTRUCT

Sufficient information should be provided on the genetic modification(s) to allow for the identification of all genetic material potentially delivered to or modified in the recipient microorganism and to provide the necessary information for the analysis of the data supporting the characterization of the DNA added to, inserted into, modified in, or deleted from the microbial genome.

The description of the strain construction process should include:

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

(2) information on the DNA used to modify the microorganism, including the source (e.g., plant, microbial, viral, synthetic), identity and expected function in the recombinant-DNA microorganism, and copy number for plasmids; and

(3) intermediate recipient organisms including the organisms (e.g., other bacteria or fungi) used to produce or process DNA prior to introduction into the final recipient organism.

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

(1) the characterization of all genetic components including marker genes, vector genes, regulatory and other elements affecting the function of the DNA;

(2) the size and identity;

(3) the location and orientation of the sequence in the final vector/construct; and

(4) the function.

4.5 CHARACTERIZATION OF THE GENETIC MODIFICATION(S)

In order to provide clear understanding of the impact of the genetic modification on the composition and safety of foods produced using recombinant-DNA microorganisms, a comprehensive molecular and biochemical characterization of the genetic modification should be carried out. To facilitate the safety assessment, the DNA to be inserted should be preferably limited to the sequences necessary to perform the intended functions.

Information should be provided on the DNA modifications in the recombinant DNA microorganism; this should include:

(1) the characterization and description of the added, inserted, deleted, or otherwise modified genetic materials, including plasmids or other carrier DNA used to transfer desired genetic sequences. This should include an analysis of the potential for mobilization of any plasmids or other genetic elements used, the locations of the added, inserted, deleted, or otherwise modified genetic materials (site on a chromosomal or extra-chromosomal location); if located on a multi-copy plasmid, the copy number of the plasmid;

(2) the number of insertion sites;

(3) the organisation of the modified genetic material at each insertion site including the copy number and sequence data of the inserted, modified, or deleted material, plasmids or carrier DNA used to transfer the desired genetic sequences, and the surrounding sequences. This will enable the identification of any substances expressed as a consequence of the inserted, modified or deleted material;

(4) identification of any open reading frames within inserted DNA, or created by the modifications to contiguous DNA in the chromosome or in a plasmid, including those that could result in fusion proteins; and

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

Information should be provided on any expressed substances in the recombinant-DNA microorganism; this should include:

(1) the gene product(s) (e.g., a protein or an untranslated RNA) or other information such as analysis of transcripts or expression products to identify any new substances that may be present in the food;

(2) the gene product’s function;

(3) the phenotypic description of the new trait(s);

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

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

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

In addition, information should be provided:

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

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

(3) to demonstrate whether the intended effect of the modification has been achieved and that all expressed traits are expressed and inherited in a manner that is stable for the extent of propagation needed for its use(s) in food production and is consistent with laws of inheritance. It may be necessary to examine the inheritance of the inserted or modified DNA or the expression of the corresponding RNA if the phenotypic characteristics cannot be measured directly;

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

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

(6) to confirm the identity and expression pattern of any new fusion proteins.

4.6 SAFETY ASSESSMENT

The safety assessment of the modified microorganism should be performed on a case by case basis depending on the nature and extent of the introduced changes. Conventional toxicology studies may not be considered necessary where the substance or a closely related substance has, taking into account its function and exposure, been consumed safely in food. In other cases, the use of appropriate conventional toxicology or other studies on the new substance may be necessary. Effects of the recombinant-DNA microorganism on the food matrix should be considered as well. If the characterisation of the food indicates that the available data are insufficient for a thorough safety assessment, properly designed animal or *in vitro* studies with the recombinant-DNA microorganism and/or the food produced using it could be considered necessary.

4.6.1 Expressed Substances: Assessment of Potential Toxicity and Other Traits Related to Pathogenicity

When a substance is new to foods or food processing, the use of conventional toxicology studies or other applicable studies on the new substance will be necessary. This may require the isolation of the new substance from the recombinant-DNA microorganism, the food product if the substance is secreted, or, if necessary, the synthesis or production of the substance from an alternative source, in which case the material should be shown to be structurally, functionally, and biochemically equivalent to that produced in the recombinant-DNA microorganism. Information on the anticipated exposure of consumers to the substance, the potential intake and dietary impact of the substance should be provided.

The safety assessment of the expressed substance should take into account its function and concentration in the food. The number of viable microorganisms remaining in the food

should be also determined and compared to a conventional counterpart. All quantitative measurements should be analysed using appropriate statistical techniques. Current dietary exposure and possible effects on population sub-groups should also be considered.

(1) In the case of proteins, the assessment of potential toxicity should take into account the structure and function of the protein and should focus on amino acid sequence similarity between the protein and known protein toxins and anti-nutrients (e.g., protease inhibitors, siderophores) as well as stability to heat or processing and to degradation in appropriate representative gastric and intestinal model systems. Appropriate oral toxicity studies may be carried out in cases where the protein is present in the food, but is not closely similar to proteins that have been safely consumed in food, and has not previously been consumed safely in food, and taking into account its biological function in microorganisms where known.

(2) Potential toxicity of non-protein substances that have not been safely consumed in food should be assessed in a case-by-case basis depending on the identity, concentration, and biological function of the substance and dietary exposure. The type of studies to be performed may include evaluations of metabolism, toxicokinetics, chronic toxicity/ carcinogenicity, impact on reproductive function, and teratogenicity.

The newly expressed or altered properties should be shown to be unrelated to any characteristics of donor organisms that could be harmful to human health. Information should be provided to ensure that genes coding for known toxins or anti-nutrients present in the donor organisms are not transferred to recombinant-DNA microorganisms that do not normally express those toxic or anti-nutritious characteristics.

(3) Additional *in vivo* or *in vitro* studies may be needed on a case-by-case basis to assess the toxicity of expressed substances, taking into account the potential accumulation of any substances, toxic metabolites or antibiotics that might result from the genetic modification.

4.6.2 Compositional Analyses of Key Components

Analyses of concentrations of key components[6/] of foods produced by recombinant-DNA microorganisms should be compared with an equivalent analysis of a conventional counterpart produced under the same conditions. The statistical significance of any observed differences should be assessed in the context of the range of natural variations for that parameter to determine its biological significance. Ideally, the comparator(s) used in this assessment should be food produced using the near isogenic parent strain. The purpose of this comparison, in conjunction with an exposure assessment as necessary, is to establish that substances that can affect the safety of the food have not been altered in a manner that would have an adverse impact on human health.

4.6.3 Evaluation of Metabolites

Some recombinant-DNA microorganisms may be modified in a manner that could result in new or altered levels of various metabolites in foods produced using these organisms. Where altered metabolite levels are identified in foods, consideration should be given to the potential

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[6/] The Joint FAO/WHO Expert Committee on Food Additives (JECFA) is revising guidelines for General Specifications and Considerations for Enzyme Preparations used in food processing. These guidelines have been used to evaluate enzyme preparations derived from genetically modified microorganisms.

impacts on human health using conventional procedures for establishing the safety of such metabolites (e.g., procedures for assessing the human safety of chemicals in foods).

New or altered levels of metabolites produced by a recombinant-DNA microorganism may change the population of microorganisms in mixed culture, potentially increasing the risk for growth of harmful organisms or accumulation of harmful substances. Possible effects of genetic modification of a microorganism on other microorganisms should be assessed when a mixed culture of microorganisms is used for food processing, such as for production of natural cheese, miso, soy sauce, etc.

4.6.4 Effects of Food Processing

The potential effects of food processing, including home preparation, on foods produced using recombinant-DNA microorganisms should also be considered. For example, alterations could occur in the heat stability of an endogenous toxicant or the bioavailability of an important nutrient after processing. Information should therefore be provided describing the processing conditions used in the production of a food. For example, in the case of yoghurt, information should be provided on the growth of the organism and culture conditions.

4.6.5 Assessment of Immunological Effects

When the protein(s) resulting from an inserted gene is present in the food, it should be assessed for its potential to cause allergy. The likelihood that individuals may already be sensitive to the protein and whether a protein new to the food supply will induce allergic reactions should be considered. A detailed presentation of issues to be considered is presented in Thai Agricultural Standard Assessment of Possible Allergenicity (TAS 9011).

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

Recombinant-DNA microorganisms that remain viable in foods may interact with the immune system in the gastrointestinal tract. Closer examination of these interactions will depend on the types of differences between the recombinant-DNA microorganism and its conventional counterpart.

4.6.6 Assessment of Viability and Residence of Microorganisms in the Human Gastrointestinal Tract

In some foods produced using recombinant-DNA microorganisms, ingestion of these microorganisms and their residence may have an impact on the human intestinal tract. The need for further testing of such microorganisms should be based on the presence of their conventional counterpart in foods, and the nature of the intended and unintended effects of genetic modifications. If processing of the final food product eliminates viable microorganisms (by heat treatment in baking bread, for example), or if accumulations of end-products toxic to the microorganism (such as alcohol or acids) eliminate viability, then viability and residence of microorganisms in the alimentary system need no examination.

For applications in which recombinant-DNA microorganisms used in production remain viable in the final food product, (for example, organisms in some dairy products), it may be desirable to demonstrate the viability (or residence time) of the microorganism alone and within the respective food matrix in the digestive tract and the impact on the intestinal microflora in appropriate systems. The nature of intended and unintended effects of genetic

modification and the degree of differences from the conventional counterpart will determine the extent of such testing.

4.6.7 Antibiotic Resistance and Gene Transfer

In general, traditional strains of microorganisms developed for food processing uses have not been assessed for antibiotic resistance. Many microorganisms used in food production possess intrinsic resistance to specific antibiotics. Such properties need not exclude such strains from consideration as recipients in constructing recombinant-DNA microorganisms. However, strains in which antibiotic resistance is encoded by transmissible genetic elements should not be used where such strains or these genetic elements are present in the final food. Any indication of the presence of plasmids, transposons, and integrons containing such resistance genes should be specifically addressed.

Alternative technologies, demonstrated to be safe, that do not rely on antibiotic resistance marker genes in viable microorganisms present in foods should be used for selection purposes in recombinant-DNA microorganisms. In general, use of antibiotic resistance markers for constructing intermediate strains should pose no significant hazards that would exclude the use of the ultimate strains in food production, provided that the antibiotic resistance marker genes have been removed from the final construct.

Transfer of plasmids and genes between the resident intestinal microflora and ingested recombinant-DNA microorganisms may occur. The possibility and consequences of gene transfer from recombinant-DNA microorganisms and food products produced by recombinant-DNA microorganisms to gut microorganisms or human cells should also be considered. Transferred DNA would be unlikely to be maintained in the absence of selective pressure. Nevertheless, the possibility of such events cannot be completely discounted.

In order to minimize the possibility of gene transfer, the following steps should be considered:

(1) chromosomal integration of the inserted genetic material may be preferable to localization on a plasmid;

(2) where the recombinant-DNA microorganism will remain viable in the gastrointestinal tract, genes should be avoided in the genetic construct that could provide a selective advantage to recipient organisms to which the genetic material is unintentionally transferred; and

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

4.6.8 Nutritional Modification

Section 4.6.2 on Compositional analyses of key components described the assessment of possible compositional changes to key nutrients, which should be conducted for all foods produced using recombinant-DNA microorganisms, has already been addressed under ‘Compositional analyses of key components.’ If such nutritional modifications have been implemented, the food should be subjected to additional testing to assess the consequences of the changes and whether the nutrient intakes are likely to be altered by the introduction of such foods into the food supply.

Information about the known patterns of use and consumption of a food and its derivatives should be used to estimate the likely intake of the food produced using the recombinant-DNA microorganism. The expected intake of the food should be used to assess the nutritional implications of the altered nutrient profile both at customary and maximal levels of

consumption. Basing the estimate on the highest likely consumption provides assurance that the potential for any undesirable nutritional effects will be detected. Attention should be paid to the particular physiological characteristics and metabolic requirements of specific population groups such as infants, children, pregnant and lactating women, the elderly and those with chronic diseases or compromised immune systems. Based on the analysis of nutritional impacts and the dietary needs of specific population subgroups, additional nutritional assessments may be necessary. It is also important to ascertain to what extent the modified nutrient is bioavailable and remains stable with time, processing, and storage.

The use of modern biotechnology to change nutrient levels in foods produced using microorganisms could result in broad changes to the nutrient profile. The intended modification in the microorganism could alter the overall nutrient profile of the product, which, in turn, could affect the nutritional status of individuals consuming the food. The impact of changes that could affect the overall nutrient profile should be determined.

When the modification results in a food product with a composition that is significantly different from its conventional counterpart, it may be appropriate to use additional conventional foods or food components (i.e., foods whose nutritional composition is closer to that of the food produced using the recombinant-DNA microorganism) as appropriate comparators to assess the nutritional impact of the food.

Some foods may require additional testing. For example, animal-feeding studies may be warranted for foods produced using recombinant-DNA microorganisms if changes in the bioavailability of nutrients are expected or if the composition is not comparable to conventional foods. Also, foods designed for health benefits, may require an assessment beyond the scope of these guidelines such as specific nutritional, toxicological or other appropriate studies. If the characterization of the food indicates that the available data are insufficient for a thorough safety assessment, properly designed animal studies could be requested on the whole food.

5 REVIEW OF SAFETY ASSESSMENTS

The goal of the safety assessment is a conclusion as to whether the food produced using a recombinant-DNA microorganism is as safe as the conventional counterpart taking into account dietary impact of any changes in nutritional content or value. Nevertheless, the safety assessment should be reviewed in the light of new scientific information that calls into question the conclusions of the original safety assessment.