codex alimentarius commission

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

WORLD HEALTH ORGANIZATION

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September 2002

TO: Codex Contact Points
Interested International Organizations

FROM: Secretary, Joint FAO/WHO Food Standards Programme
FAO, 00100 Rome, Italy

SUBJECT: **Request for comments on the Draft Guideline for the Conduct of Food Safety Assessment of Foods Produced Using Recombinant-DNA Microorganisms *at Step 6***

DEADLINE: **29 November 2002**

COMMENTS:

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BACKGROUND

The 3rd Session of the Codex *Ad Hoc* Intergovernmental Task Force on Foods Derived from Biotechnology discussed the *Proposed Draft Guideline for the Conduct of Food Safety Assessment of Foods Produced Using Recombinant-DNA Microorganisms* and agreed to advance it to the next session of the Codex Executive Committee for its adoption at Step 5 since the Task Force noted that the general approach and outline of the Proposed Draft while it noted there were several proposals to amend the paragraphs in the text (para 88, ALINORM 03/34).

The 50th Session of the Codex Executive Committee adopted it at Step 5 and advanced to Step 6 *Draft Guideline for the Conduct of Food Safety Assessment of Foods Produced Using Recombinant-DNA Microorganisms*.

Therefore, the text (Appendix V in ALINORM 03/34) attached as Annex to this letter is hereby circulated for comments *at Step 6*. Governments and international organizations wishing to submit comments should do so in writing, preferably by email, to the above addresses **before 29 November 2002**.

Attention is drawn to paragraph 44 of the text which two alternative proposals on the treatment of the Annex on Allergenicity are presented.

Annex

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

(At Step 6 of the Procedure)

SECTION 1 – SCOPE

1. This Guideline supports the Principles for the Risk Analysis of Foods Derived from Modern Biotechnology and addresses safety 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 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.

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

   - safety of microorganisms used in agriculture (for plant protection, biofertilizers, in animal feed or food derived from animals fed the feed etc.);
   - risks related to environmental releases of recombinant-DNA microorganisms used in food production;
   - safety of substances produced by microorganisms that are used as additives or processing aids, including enzymes for use in food production;[3]
   - specific purported health benefits or probiotic effects that may be attributed to the use of microorganisms in food; or
   - issues relating to the safety of food production workers handling recombinant-DNA microorganisms.

3. 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. Microorganisms are amenable to modification using recombinant-DNA technology and new strains can be rapidly developed due to their rapid growth rates. 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

[1] The microorganisms included in these applications are bacteria, yeasts, and filamentous fungi. (Such uses include, but are not limited to, production of yogurt, cheese, fermented sausages, natto, kimchi, bread, beer, and wine.)

[2] The criterion for establishing the safety of microorganisms used in the production of foods where there is no history of safe use is beyond the scope of the current document.

[3] The Working Group noted that the Joint FAO/WHO 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.

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 organisms and the absence of reports of adverse events attributed to ingestion of these organisms, 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, an alternative approach is required where the safety of a whole food is being considered.

4. Information considered in developing this approach includes:

   A)uses of living microorganisms in food production;

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

   C)the types of methodologies available for performing a safety assessment;

   D)issues specific to microorganisms used in food production, including their genetic stability, gene transfer, colonization of the intestinal tract and persistence therein and, interactions with the recombinant-DNA microorganism, the gastrointestinal flora and the mammalian host, and impacts on the immune system.

5. 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.

6. This safety assessment approach falls within the risk assessment framework as discussed in Section 3 of the Principles for the Risk Analysis of Foods Derived from Modern Biotechnology. 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, 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 the Principles for the Risk Analysis of Foods Derived from Modern Biotechnology before it is considered for commercial distribution.

7. 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, [or] 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. 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. [On the condition that the microorganism is considered to be safe when compared with the conventional counterpart taking into account its interactions with the food matrix or the microflora, that any newly expressed protein(s) encoded by the modified DNA is considered to be safe, and that any secondary metabolic products present as a consequence of the genetic modifications are deemed to be safe, it is unlikely that the food produced by the microorganism would be harmful to human health.]

SECTION 2 – DEFINITIONS

8. The definitions below apply to this Guideline**:**

*"*Recombinant-DNA Microorganism*"* **-** means bacteria, yeasts or filamentous fungi in which the genetic material has been changed through in vitro nucleic acid techniques [4] including recombinant deoxyribonucleic acid (DNA) and direct injection of nucleic acid into cells or organelles.

"**Conventional Counterpart**"[5] – means:

- a microorganism/strain used for food production or processing related to the recombinant-DNA strainwith a known history of safe use in producing the food to be produced by the recombinant-DNA microorganism. 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.

SECTION 3 - INTRODUCTION TO FOOD SAFETY ASSESSMENT

9. 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.

10. 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, the effects and safety of the food should be examined.

11. 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.

[4] These include but are not limited to: recombinant-DNA techniques that use vector systems and techniques involving the direct introduction into the organism of hereditary materials prepared outside the organism such as microinjection, macroinjection, chemoporation, electroporation, microencapsulation, and liposome fusion.

[5] It is recognized that for the foreseeable future, microorganisms derived from modern biotechnology will not be used as conventional counterparts.

12. 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. 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.

13. 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. 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.

14. Due to the difficulties of applying traditional toxicological testing and risk assessment procedures to whole foods produced using microorganisms, an alternative approach is required for the safety assessment of foods produced using microorganisms, including 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*[5]. 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 [as well as the food produced with the aid of the RDM relative to its conventional counterpart]. This concept is used to identify similarities and differences between a recombinant-DNA microorganism used in food processing and its conventional counterpart. Generally, the comparison should be between the recombinant-DNA microorganism and its recipient strain used in its development. [An evaluation of the differences between the recombinant-DNA microorganism and its conventional counterpart could be a starting point to address safety concerns.] However, there will be instances when the food or specific gene product(s) encoded by the modified DNA and produced by the recombinant DNA microorganism should be compared with the appropriate conventional counterpart. 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 can be considered relative to its conventional counterpart.

UNINTENDED EFFECTS

15. 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. Such unanticipated changes are referred to as unintended effects. The potential for occurrence of unintended effects is not restricted to the use of *in*

[5] The concept of *substantial equivalence* as described in FAO /WHO Expert Consultation on Foods Derived from Biotechnology- Safety aspects of genetically modified plants, 29 May – 2 June, 2000, Geneva, Switzerland, and Section 4.3 of the Joint FAO/Who Expert Consultation of Foods Derived from Biotechnology,- Safety assessment of foods derived from genetically modified microorganisms, 24-28 September, 2001, Geneva, Switzerland.

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.]

16. 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.

17. 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.

18. 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.

19. 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.

FRAMEWORK OF FOOD SAFETY ASSESSMENT

20. 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:

    A)Description of the recombinant-DNA microorganism;

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

    C)Description of the donor organism(s);

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

E)Characterization of the genetic modification(s);

F)Safety assessment:

a. expressed substances including toxins or other traits related to pathogenicity (e.g., adhesins, invasins);

b. compositional analyses of key components;

c. evaluation of metabolites;

d. effects of food processing;

e. assessment of immunological effects;

f. assessment of viability, viable population and residence of microorganisms in the human gut;

g. antibiotic resistance and gene transfer; and,

h. nutritional modification .

21. In certain cases, the characteristics of the microorganisms may necessitate generation of additional data and information to address issues that are unique to the product under review.

22. 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.

23. 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 is as safe as the conventional counterpart taking into account dietary impact of any changes in nutritional content or value. Where the microorganism is likely to be viable upon ingestion, the safety of the microorganism should be compared to a conventional counterpart taking into account residence of the recombinant-DNA microorganism in the GI tract. 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 and if so to make well-informed and appropriate decisions.

SECTION 4- GENERAL CONSIDERATIONS

DESCRIPTION OF THE RECOMBINANT-DNA MICROORGANISM

24. 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 intended differences in the nature of the organism or food produced using the organism being submitted for safety assessment[All recombinant-DNA microorganisms should be deposited into an international culture collection with appropriate identification using modern molecular methods.]

DESCRIPTION OF THE RECIPIENT MICROORGANISM AND ITS USE IN FOOD PRODUCTION

25. 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, or that are likely to contain genes conferring functions associated with pathogenicity (i.e., also known as pathogenicity islands or virulence factors) should not be considered for use as recipients. The necessary data and information should include, but need not be restricted to:

A) 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;

B) 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;

C) information on the recipient microorganism's genotype and phenotype relevant to its safety, including any known toxins, other factors related to pathogenicity, or immunological impact, and information about the genetic stability of the microorganism; and

D) history of safe use in food production.

26. Relevant phenotypic and genotypic information should be provided not only for the recipient microorganism, but also for related species and for any extrachromosomal 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 when available including the presence of mobile DNA elements, i.e. insertion sequences, transposons, plasmids, and prophages.

27. 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.

DESCRIPTION OF THE DONOR ORGANISM

28. 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:

A) 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, and information supporting its taxonomic assignment;

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

C) information on the microorganisms' genotype and phenotype relevant to its safety including any known toxins, other factors related to pathogenicity, or immunological impact;

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

E) information on opportunistic pathogenicity.

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

29. Sufficient information should be provided on the genetic modification(s) to allow for the identification of

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.

30. The description of the strain construction process should include:

    A)information on the specific method(s) used for genetic modification[6];

    B)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

    C)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.

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

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

    B)the size and identity;

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

    D)the function.

CHARACTERIZATION OF THE GENETIC MODIFICATION (S)

32. 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 limited to the sequences necessary to perform the intended functions.

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

    A)the characterization and description of the added, inserted, deleted, or otherwise modified genetic materials, including plasmids or other carrier DNA used to transfer desired genetic sequences. This should include 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 extrachromosomal location); if located on a multicopy plasmid, the copy number of the plasmid;

    B)the number of insertion sites;

    C)the organization of the modified genetic material at each insertion site, including copy number, if applicable. Sequence data of the inserted material and of the surrounding region should be provided in electronic format to facilitate of analysis using sequence databases;

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

    E)particular reference to any sequences known to encode potentially harmful functions.

34. Information should be provided on any expressed substances in the recombinant-DNA microorganism;

[6] General mechanisms of genetic exchange have been specified in footnote 4. Mobile promoter elements or virus-mediated exchange events and processes may not yet be available but are equally as valid as the general categories listed.

this should include, when applicable:

A)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;

B)the gene product's function;

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

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

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

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

35. In addition, information should be provided:

A)to demonstrate whether the arrangement of the modified genetic material has been conserved[7] 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;

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

C)to demonstrate whether the intended effect of the modification has been achieved and that all expressed traits are expressed and inherited in a manner that is stable for the extent of propagation needed for its use(s) in food production and is consistent with laws of inheritance. It may be necessary to examine the inheritance of the inserted or modified DNA or the expression of the corresponding RNA if the phenotypic characteristics cannot be measured directly;[8]

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

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

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

SAFETY ASSESSMENT

[36.*In vitro* nucleic acid techniques enable the introduction of new DNA to cells or enable precise changes to DNA in cells, which can result in the synthesis of new substances in or by microorganisms, alterations to the substances produced by microorganisms, or the regulation of these substances. Methods for implementing precise genetic changes are readily available for application to microorganisms and DNA is easily integrated into microbial genomes. These can be normal cellular components such as proteins, fats, carbohydrates, or other compounds such as vitamins or metabolites that are not normally present or produced by the recipient organism. Conventional toxicology studies may not be considered necessary where the substance or a closely related substance has been consumed safely in food or used in food

[7] Microbial genomes are more fluid than those of higher eukaryotes; that is, the organisms grow faster, adapt of changing environments, and are more prone to change. Chromosomal rearrangements are common. The general genetic plasticity of microorganisms may affect recombinant DNA in microorganisms and must be considered in evaluating the stability of recombinant DNA microorganisms.

[8 Modified strains should be maintained by successive subculture or new culture to be used in an uninterrupted way during the successive productions in order to verify the genetic stability.]

processing, taking into account its function and exposure. Effects of the recombinant-DNA microorganisms on the food matrix should be considered.]

Expressed Substances Including Toxins or Other Traits Related to Pathogenicity

37. 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 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.

38. 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, compared to a conventional counterpart. All quantitative measurements should include variations and mean values. Current dietary exposure and possible effects on population sub-groups should also be considered.

    - In the case of proteins, the assessment of potential toxicity 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[9] may be carried out in cases where the protein is present in the food, but is not 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.
    - 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.

39. 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.

    - 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.

Compositional Analyses of Key Components

40. Analyses of concentrations of key components [10] 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,

[9] Guidelines for oral toxicity studies have been developed in international fora, for example the OECD Guidelines for the Testing of Chemicals.
[10] Key nutrients or key anti-