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-nutrients are those components in a particular food that may have a substantial impact in the overall diet. They may be major nutritional constituents (fats, proteins, carbohydrates), enzyme inhibitors as anti-nutrients, or minor compounds (minerals, vitamins). Key toxicants are those toxicologically significant compounds known to be produced by the microorganism, such as those compounds whose toxic potency and level may be significant to health. Microorganisms traditionally used in food processing are not usually known to produce such compounds under production conditions.

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.

Evaluation of Metabolites

41. 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 residue or metabolite levels are identified in foods, consideration should be given to the potential 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).

42. 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.

Effects of Food Processing

43. 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.

Assessment of immunological effects

44. 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 [an annex for the proposed draft guideline for the conduct of food safety assessment of foods derived from recombinant-DNA plants[11]][in the Annex to this guideline].

45. The transfer of genes from species that are commonly allergenic when ingested as food should be avoided, unless the proteins associated with allergy from those species have been identified and do not include the protein encoded by the transferred gene.

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

Assessment of Viability and Residence of Microorganisms in the Human Gut

47. In some foods produced using recombinant-DNA microorganisms, ingestion of these microorganisms and their residence[12] may have an impact on the human intestinal tract. The need for further testing of

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[11] Codex *Draft Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants* (under development at Step 7) including the *Proposed Draft Annex on the Assessment of Possible Allergenicity of the Draft Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants* (under development at Step 4).

[12] Permanent life-long colonization by ingested microorganisms is rare. Some orally administered microorganisms have been recovered in faeces or in the colonic mucosa weeks after feeding ceased. Residence connotes survival of microorganisms in the GI tract longer than two intestinal transit times (International Life Science Institute, *The safety assessment of viable genetically modified microorganisms used as food,* 1999, Brussels; WHO/FAO Joint Expert Consultation on Foods Derived from Biotechnology- *Safety assessment of foods*

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 endproducts toxic to the microorganism (such as alcohol or acids) eliminate viability, then viability and residence of microorganisms in the alimentary system need no examination.

48. 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 of the microorganism in the digestive tract in animal model systems or to establish the residence times for the microorganisms in the alimentary tract and how dose affects other microorganisms in the alimentary system] /[it is desirable to demonstrate the viability and colonization of the microorganism in the digestive tract as well as how dose affect other microorganisms in the alimentary system]/[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 should be examined in appropriate systems.] [The nature of the intended effects and the degree of differences from the conventional counterpart will determine the extent of such testing.]

Antibiotic Resistance and Gene Transfer

49. 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 with transmissible antibiotic resistance should be avoided [when such a resistance is present in genetic elements]as candidate recipients for constructing recombinant-DNA strains. The absence of plasmids, transposons, and integrons containing such resistance genes should be[verified].

50. 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.

51. 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.

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

- chromosomal integration of the inserted genetic material may be preferable to localization on a plasmid;
- genes that could provide a selective advantage [,under the condition in which the recombinant microorganisms is used in the food production and stays viable in the human GI tract after its consumption,] should be avoided in constructing the introduced genetic material; and,
- sequences that mediate integration into other genomes should be avoided in constructing the introduced genetic material.

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derived from genetically modified microorganisms, 24-28 September, 2001, Geneva, Switzerland).

Nutritional Modification

53. 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 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.

54. 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.

55. 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.

56. 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.

57. 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 foods.

REVIEW OF SAFETY ASSESSMENTS

58. 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.