37. 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, including those that may occur during its storage according to current techniques;

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 more 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

38. 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.

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

39. 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.

[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 in a manner to enable verification of the genetic stability.

40. 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.

    - 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[9] 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.
    - 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.

41. 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

42. 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, 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

43. 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 impacts on human health

[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.

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

44. 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

45. 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

46. 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 the Annex to this guideline.

47. 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.

48. 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.

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

49. In some foods produced using recombinant-DNA microorganisms, ingestion of these microorganisms and their residence[11] 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 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.

[11] 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. Whether the genetically modified microorganism is established in the gastrointestinal tract or not, the possibility remains that it might influence the microflora or the mammalian host (Joint FAO/WHO Expert Consultation on Foods Derived from Biotechnology – *Safety assessment of foods derived from genetically modified microorganism*, 24-28 September, 2001, Geneva, Switzerland).