

Appendix II

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

(At Step 8 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 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.
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. 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

¹ The microorganisms included in these applications are bacteria, yeasts, and filamentous fungi. (Such uses could 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 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.

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.

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; and
 - D) 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 persistence⁴ therein, interactions that the recombinant-DNA microorganism may have with the gastrointestinal flora or the mammalian host, and any impact of the recombinant-DNA microorganism 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. Risk management measures such as post-market monitoring of consumer health effects may assist the risk assessment process. These are discussed in paragraph 20 of the Draft Principles for the Risk Analysis of Foods derived from Modern Biotechnology.
8. 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

⁴ Persistence connotes survival of microorganisms in the gastrointestinal tract longer than two intestinal transit times (International Life Science Institute, *The safety assessment of viable genetically modified microorganisms used as food*, 1999, Brussels; the Joint FAO/WHO Expert Consultation on Foods Derived from Biotechnology- *Safety assessment of foods derived from genetically modified microorganisms*, 24-28 September, 2001, Geneva, Switzerland).

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.

SECTION 2 – DEFINITIONS

9. 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 including recombinant deoxyribonucleic acid (DNA) and direct injection of nucleic acid into cells or organelles.

“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.

SECTION 3 - INTRODUCTION TO FOOD SAFETY ASSESSMENT

10. 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.

11. 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.

12. 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

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

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.

13. 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.
14. 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.
15. 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*⁶.
16. 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 microorganism used in food processing as well as the food

⁶ The concept of *substantial equivalence* as described in the Joint 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.

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.

Unintended Effects

17. 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.
18. 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.
19. 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.
20. 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.
21. 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,