

## 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<sup>4</sup> including recombinant deoxyribonucleic acid (DNA) and direct injection of nucleic acid into cells or organelles.

“Conventional Counterpart”<sup>5</sup> – means:

- a microorganism/strain used for food production or processing related to the recombinant-DNA strain with 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.

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<sup>4</sup> 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.

<sup>5</sup> 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*<sup>5</sup>. 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*

<sup>5</sup> 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.

*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.]

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);