

### 3. The comparative approach for safety assessment of foods derived from recombinant-DNA plants

#### Introduction

To date, the safety assessment of foods derived from recombinant-DNA plants has been based on the principle that these products can be compared with conventional counterparts that have an established history of safe use. The objective is to determine if the food presents any new or altered hazard in comparison with its conventional counterpart. The goal is not to establish an absolute level of safety, but the food should be as safe as its conventional counterpart in the sense that there is a reasonable certainty that no harm will result from its intended use under the anticipated conditions of processing and consumption.

#### Principles of the comparative approach

Accounting for processing and consumption patterns is important even for conventional foods. A number of plants consumed by humans are acutely toxic in their raw state, but are accepted as food because processing methods alter or eliminate this toxicity. For example, the cassava root is quite toxic, but proper processing converts it into a nutritious and widely consumed food. Soybeans and lima beans, among other crops, contain antinutrients (e.g. soybean trypsin inhibitor and lectins) and require proper processing. Potatoes and tomatoes can contain toxic levels of the glycoalkaloids solanine and alpha-tomatine, respectively. Thus, the presence of a toxicant in a plant variety does not necessarily eliminate its use as a food source. In considering the safety of the food derived from recombinant-DNA plants, it is therefore important to examine the range of possible toxicants, critical nutrients and other relevant factors, as well as its processing, intended use and exposure levels. The choice of compounds to be analysed is based on experience gained with conventional crops, and the OECD Task Force for the Safety of Novel Foods and Feed has developed a number of internationally agreed Consensus Documents that provide guidance on the particular compounds that should be analysed.

The comparative approach has been embodied in the concept of substantial equivalence – a concept that was developed before foods derived from modern biotechnology came to the market. The concept was first described in an OECD publication in 1993 (OECD, 1993). This document was developed by some 60 experts from 19 OECD countries, who spent more than two years discussing how to assess the safety of foods derived from modern biotechnology. The concept of substantial equivalence was further endorsed by an FAO/WHO Joint Expert Consultation in 1996. This consultation recognized that the establishment of substantial equivalence is not an assessment of safety per se, but that it gives structure to the safety analysis of the characteristics and composition of food derived from recombinant-DNA plants. Establishing equivalence to a conventional food with a history of safe consumption indicates that the new product will be as safe as the conventional food under similar consumption patterns and processing practices.

One important benefit of the concept of substantial equivalence is that it provides flexibility, which can be useful in the safety assessment of food derived from modern biotechnology. It is a

tool that helps to identify any difference, deliberate or unintended, which might be the focus of further safety evaluation. Because it facilitates a comparative process for evaluating safety, the concept of substantial equivalence can be applied at several points along the food chain (e.g. at the level of the harvested or unprocessed food product, the individual processed fractions, or the final food product or ingredient). This allows the safety assessment to be targeted to the most appropriate level based upon the nature of the product under consideration.

The Joint FAO/WHO Expert Consultation on Food Derived from Biotechnology – Safety Aspects of Genetically Modified Foods of Plant Origin (FAO/WHO, 2000) re-examined the concept of substantial equivalence and concluded that the safety assessment requires an integrated stepwise case-by-case approach, which can be aided by a structured series of questions. They reaffirmed that the concept of substantial equivalence, which focuses on the determination of similarities and differences between the foods derived from recombinant-DNA plants and their conventional counterparts and aids in the identification of potential safety and nutritional issues, and that this comparative approach is the most appropriate strategy for evaluating the safety and nutritional quality of foods derived from recombinant-DNA plants. They further clarified that the concept of substantial equivalence is not a safety assessment in itself as it does not characterize hazard; rather it should be used to structure the safety assessment of a food derived from a recombinant-DNA plant relative to its conventional counterpart (the comparator). The consultation was satisfied with the approach used to assess the safety of foods derived from recombinant-DNA plants that have been approved for commercial use. The consultation concluded that the application of the substantial equivalence concept contributes to a robust safety assessment framework. In fact, there are currently no alternative strategies that provide a better assurance of safety (FAO/WHO, 2000).

The Codex Guideline includes the reference to substantial equivalence (paragraph 13). Note that wherever text from the Codex Guideline is referenced, it is identified by both a box and a reference to the relevant paragraphs of the Guideline (Appendix 2).

**CODEx GUIDELINE PARAGRAPH 13.** The concept of substantial equivalence is a key step in the safety assessment process. However, it is not a safety assessment in itself; rather it represents the starting point which is used to structure the safety assessment of a new food relative to its conventional counterpart<sup>7</sup>. This concept is used to identify similarities and differences between the new food and its conventional counterpart. 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 derived from recombinant-DNA plants. 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 new product can be considered relative to its conventional counterpart.

<sup>7</sup> The concept of *substantial equivalence* as described in the report of the 2000 joint FAO /WHO expert consultations (Document WHO/SDE/PHE/FOS/00.6, WHO, Geneva, 2000).

## Identifying unintended effects

The applicability of the substantial equivalence concept in the safety assessment of recombinant-DNA plants has been questioned (Millstone *et al.*, 1999). However, the utility of the concept is well established, and several expert consultations (FAO/WHO, 1996, 2000) have found that safety assessments based on the concept of substantial equivalence are the most practical approach developed to date to address the safety of foods developed through modern biotechnology. Equivalence can be established relatively easily when the new gene product is targeted and can be utilized directly without resulting in any further modification to the existing metabolic pathways of the plant. However, the changes in recombinant-DNA derived plants and food sometimes may not be reflected in the known compounds that are preselected for equivalence assessment, due to unintended changes resulting from insertion of the new gene. In such cases, non-targeted profiling approaches will be essential to identify any unintended effects that are not predictable. Genomic strategies using bioinformatics tools can be effective in analysing unintended changes occurring at the RNA transcript, amino acid, protein or metabolic levels (Stiekema and Nap, 2004). Paragraphs 14 to 17 of the Codex Guidelines specifically address unintended changes.

**CODEX GUIDELINE PARAGRAPH 14.** In achieving the objective of conferring a specific target trait (intended effect) to a plant by the insertion of defined DNA sequences, additional traits could, in some cases, be acquired or existing traits could be lost or modified (unintended effects). The potential 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 conventional breeding. Unintended effects may be deleterious, beneficial, or neutral with respect to the health of the plant or the safety of foods derived from the plant. Unintended effects in recombinant-DNA plants may also arise through the insertion of DNA sequences and/or they may arise through subsequent conventional breeding of the recombinant-DNA plant. Safety assessment should include data and information to reduce the possibility that a food derived from a recombinant-DNA plant would have an unexpected, adverse effect on human health.

**CODEX GUIDELINE PARAGRAPH 15.** Unintended effects can result from the random insertion of DNA sequences into the plant genome which may cause disruption or silencing of existing genes, activation of silent genes, or modifications in the expression of existing genes. Unintended effects may also result in the formation of new or changed patterns of metabolites. For example, the expression of the enzymes at high levels may give rise to secondary biochemical effects or changes in the regulation of metabolic pathways and/or altered levels of metabolites.

**CODEX GUIDELINE PARAGRAPH 16.** Unintended effects due to genetic modification may be subdivided into two groups: those that are "predictable" and those that are "unexpected". Many unintended effects are largely predictable based on knowledge of the inserted trait and its metabolic connections or of the site of insertion. Due to the expanding information on plant genome and the increased specificity in terms of genetic materials introduced through recombinant-DNA techniques compared with other forms of plant breeding, it may become easier to predict unintended effects of a particular modification. Molecular biological and biochemical techniques can also be used to analyse potential changes at the level of gene transcription and message translation that could lead to unintended effects.

**CODEX GUIDELINE PARAGRAPH 17.** The safety assessment of foods derived from recombinant-DNA plants 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 are 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, provide assurance that the food is unlikely to have an adverse effect on human health. The assessment for unintended effects takes into account the agronomic/phenotypic characteristics of the plant that are typically observed by breeders in selecting new varieties for commercialization. These observations by breeders provide a first screen for plants that exhibit unintended traits. New varieties that pass this screen are subjected to safety assessment as described in Sections 4 and 5.

## Some examples of substantial equivalence tests

As the following examples demonstrate, new products with intentionally altered nutritional profiles will challenge our ability to assess unintended consequences. The first example relates to genetically engineered low-glutelin rice, which has been created by introducing the glutelin-encoding gene in the antisense orientation, for commercial production of sake. The decrease in glutelin level was associated with an unintended increase in the level of prolamins. The change in prolamin level was not detected by standard nutritional analyses, such as total protein and amino acid profiles, but was only observed following sodium dodecylsulphate (SDS) polyacrylamide gel electrophoresis (PAGE). While the change in prolamin level did not affect the industrial application, it could affect nutritional quality and allergenic potential if the rice were used as a food. A second example relates to genetically engineered "Golden Rice" designed to express increased levels of beta-carotene, a precursor to vitamin A. Unexpectedly, it was found that this modification was accompanied by higher levels of xanthophylls, a change that would not have been apparent from standard nutritional analyses but was detected from high-pressure liquid chromatography (HPLC) analyses for carotenoids. As these two examples illustrate, targeting a single nutrient of a complex metabolic pathway can lead to unintended alterations in the levels of other constituents, and specialized analytical methodologies may be required to assess changes in the overall nutrient profile.

Another consequence of the introduction of significant nutritional changes in a food may be the requirement for post-market monitoring of this food. In such cases, the primary objective

would be to determine if the patterns of dietary intake are altered by the introduction of the food to the market.

## Substantial equivalence – issues of concern in its application

The substantial equivalence concept is used to structure the safety assessment and to identify similarities and differences between the new food and its conventional counterpart. It is recognized that the substantial equivalence is not a safety assessment in itself, nor is it an endpoint but just a starting point for the safety assessment (FAO/WHO, 2000). The following points should be considered when adopting the substantial equivalence approach.

First, the concept depends on the presence of a relevant comparator and on the information that is available or can be generated for the comparator. The choice of comparator is therefore crucial to effective application of the concept. The comparator must have a well documented history of safe use. If adverse effects have been associated with the particular food type, specific components that are considered to cause these effects should be described and well characterized to permit effective comparison. Establishing a baseline for comparative analyses can be challenging if the recombinant-DNA plant is developed for cultivation under conditions of stress that are non-permissive for growth of the conventional counterpart.

Second, the plant-specific and relevant parameters that should be compared to establish substantial equivalence must be identified on a case-by-case basis because there is a possibility that unintended compositional changes may be overlooked in the comparative approach.

Third, the inherent variability in most parameters measured in biological systems can make interpretation of the significance of observed changes difficult. A comparative approach therefore relies on an accurate understanding of the baseline variation in the parameters to be compared. The choice of comparator will influence the range of the baseline data and must be carefully evaluated in relation to the relevant risk hypothesis that underlies parameter selection.

## Final remarks

Safety assessment of a whole food requires a different approach from that which has been used to assess the safety of individual chemical substances such as food additives or pesticides. Unlike individual chemical substances, whole foods are composed of a variety of compounds that contribute to their nutritional value. Foods produced from many crops also contain natural toxicants, antinutrients, and other substances that are important to the plant but which if present in sufficient quantities in the food may be harmful to humans. The Codex Guideline on recombinant-DNA plants recommends that a comparative assessment be used to determine if a food derived from a recombinant-DNA plant is as safe as an appropriate comparator food. The underlying assumption of this approach is that conventionally bred and cultivated crops have gained a history of safe use for consumers, animals and the environment. Using conventional breeding methods, developers have selected varieties of crops that each contain thousands of substances that are considered overall to be safe for human consumption.

## References

- FAO/WHO. 1996. Biotechnology and food safety, FAO/WHO consultation 30 Sept–4 Oct 1996. Food and Agriculture Organization, Rome and World Health Organization, Geneva.  
<http://www.fao.org/ag/agn/food/pdf/biotechnology.pdf>
- FAO/WHO. 2000. Safety aspects of genetically modified foods of plant origin, FAO/WHO consultation 29 May–2 June 2000. Food and Agriculture Organization, Rome and World Health Organization, Geneva.  
[http://www.who.int/foodsafety/publications/biotech/ec\\_june2000/en/index.html](http://www.who.int/foodsafety/publications/biotech/ec_june2000/en/index.html)

- Millstone, *et al.* 1999. Beyond substantial equivalence. *Nature*, 401: 525–526.
- OECD. 1993. Safety evaluation of foods derived by modern biotechnology, concepts and principles. Organization for Economic Co-operation and Development (OECD), Paris.
- OECD. 2000. Report of the task force for the safety of novel foods and feeds. C(2000)86/ADD1. Organization for Economic Co-operation and Development (OECD), Paris.
- Stiekema W.J. & Nap P.J. 2004. Bioinformatics for biosafety: predicting the allergenicity in GM food. In P.J. Nap, A. Atanosov & W.J. Stiekema, eds. *Genomics for biosafety in plant biotechnology*, pp. 98–116. NATO Science Series, Series I – Life and behavioral sciences, Vol 359. Amsterdam, IOS Press.
- United States National Academy of Sciences. 2004. Safety of genetically engineered foods: approaches to assessing unintended health effects. Washington, DC, The National Academies Press.
- World Bank. 2003. Biosafety regulation: a review of international approaches (Report No. 26028). The World Bank Agriculture and Rural Development Department, Washington, DC.

### Additional resources

- ILSI. 2004. Nutritional and safety assessment of foods and feeds nutritionally improved through biotechnology. *Comp. Rev. Food Sci. Food Safety*, 3: 38–104.
- OECD. 2000. Genetically modified foods: widening the debate on health and safety. (updated document of “Substantial equivalence and the safety assessment of GM foods”) Organization for Economic Co-operation and Development, Paris.  
<http://www.oecd.org/dataoecd/34/30/2097312.pdf>
- WHO. 1995. Application of the principles of substantial equivalence to safety evaluation of foods or food components from plants derived by modern biotechnology. Report of a WHO Workshop. World Health Organization, Geneva. WHO/FNU/FOS/95.1.
- WHO. 2005. Modern food biotechnology, human health and development: an evidence-based study. World Health Organization, Geneva.  
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