Part One

Principles of safety assessment of food derived from recombinant-DNA plants

1. Introduction

Scope of the training package

This package was developed in this context to present a framework for the safety assessment of foods derived from recombinant-DNA plants, based on internationally accepted principles and guidance. Additionally, it introduces other issues related to the topic and provides links to useful resources. Practical information about organizing and delivering a training workshop is also included.

Several international documents are being prepared on safety assessment of genetically modified (GM) foods other than those derived from recombinant-DNA plants, and additional training materials will also be developed by FAO. This particular training package does not address the safety assessment of foods derived from other recombinant organisms (such as microorganisms and animals) or livestock feeds derived from recombinant-DNA plants, nor does it consider the ethical and socio-economic issues, and environmental risks, that may be associated with the release of recombinant-DNA plants.

Objectives

In order to support capacity building in food safety assessment, FAO, in collaboration with many international, intergovernmental and governmental bodies, has supported the development of a standardized training programme to assist countries in implementing international documents related to the risk analysis of products containing or derived from genetically modified organisms. Specifically, the training package should be used for implementation of programmes that:

- promote a harmonized international regulatory approach to countries that have requested such guidance, to ensure consistency and uniformity in the application of international standards;
- provide regulators in the beneficiary countries with information on internationally accepted approaches to the evaluation of foods derived from recombinant-DNA plants;
- endorse a transparent, science-based approach to the safe introduction and use of foods derived from recombinant-DNA plants.

Target audience and trainer qualifications

The target audience includes national food safety regulators, authorities, and/or scientists tasked with training others to undertake the safety assessment of foods derived from recombinant-DNA plants. While developed mainly for government agencies in developing countries, this tool may also be of use to agencies in developed countries, as well as to donor organizations and agencies supporting capacity building activities in food safety.

Expected qualifications for the trainer include a Ph.D. degree in natural sciences or an equivalent combination of education and experience, and extensive experience as a regulator or as a senior scientist active in a scientific area relevant to the safety assessment of GM foods. Examples of relevant areas include: molecular biology, plant breeding, biochemistry,

immunology, toxicology, and human or livestock health and nutrition. Experience with working in a multidisciplinary environment with people of different nationalities, ethnic and cultural backgrounds would be an asset. Proficiency in using computers, on-line communication and information retrieval is expected. The trainer is also expected to have in-depth knowledge of both public and private sector research and development, and to have excellent language, communication and presentation skills, particularly to different audiences. A publication record in the scientific literature or in dossier evaluation is required. Trainers should be selected on their personal capacities in a transparent manner. For international training courses attention should be paid to geographical and gender balance.

Contents of the training package

The package is composed of three parts with a CD-ROM containing the visual aids and other relevant reference materials. The first part, *Principles of safety assessment of foods derived from recombinant-DNA plants,* provides guidance text for the implementation of an effective framework for safety assessment of foods derived from recombinant-DNA plants. The second part, *Tools and techniques for trainers*, offers a practical guide for preparing and delivering a workshop on the topic of safety assessment of foods derived from recombinant-DNA plants. This section includes various checklists and forms, a sample workshop agenda, sample workshop evaluation sheet, and five useful presentation modules for trainers. All forms, presentations and copies of the relevant Codex Alimentarius documents are included in the CD-ROM in electronic format. The third part, *Case studies*, presents three safety assessment dossiers that have been summarized for training purposes[3]. All three case studies have been developed based on the data and information submitted for the food safety assessment regulatory evaluation conducted by Governmental agencies such as Health Canada, the United States Food and Drug Administration, and Food Standards Australia New Zealand. The case studies are in-kind contributions that have been provided by Agbios, Inc., Ottawa, Canada, and the Canadian Government, represented by Health Canada[4].

Expected outcomes

Upon completion of training administered using this training tool as a guide, the audience will be able to plan and deliver GM food safety assessment training for national food safety authorities, regulators, and/or scientists in their own training programmes ●

3 In order to enhance the utility of the case studies for training purposes, certain information has been summarized and the data presented in the case studies are only a subset of those actually submitted. The case studies do not reflect a complete application, nor a complete safety assessment.

4 These case studies are included in this training package without any modification or enhancement by FAO. The views expressed in the case studies do not necessarily reflect the views of FAO.

2. Concepts and principles of safety assessment of food derived from recombinant-DNA plants (within international frameworks)

Introduction

Modern biotechnology broadens the scope of genetic changes that can be introduced into organisms used for food. However, it does not inherently result in foods that are less safe than those produced by more conventional techniques (OECD, 1993; US NAS, 2004). This principle has important ramifications for the safety assessment of GM foods. It means that a new or different standard of safety is not required, and that previously established principles for assessing food safety still apply. Moreover, introducing specific genetic changes should enable a more direct and focused assessment of safety.

While countries may differ in statutory and non-statutory approaches to regulating foods derived from recombinant-DNA plants, the criteria used to assess the safety of these products is generally consistent from one country to another (World Bank, 2003). This reflects the concerted efforts that have been made internationally to harmonize the risk assessment of foods derived from modern biotechnology (Table 2.1). The outcomes of these consultations have contributed significantly to the development of internationally accepted approaches to assessing the safety of foods derived from biotechnology, as articulated in two important documents published in 2003 by the Codex Alimentarius Commission (CAC)[5]: *Principles for the Risk Analysis of Foods Derived from Modern Biotechnology* (hereinafter referred to as "Codex Principles"; see Appendix 1) and *Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants CAC GL 45-2003* (hereinafter referred to as "Codex Guideline"; see Appendix 2).

These documents acknowledge the inadequacy of applying already established risk assessment principles to foods, which by nature are complex compounds and not single chemicals that can be investigated individually. Nevertheless, the documents describe the safety assessment of foods derived from recombinant-DNA plants as a process within the established framework of risk assessment. Safety assessment is in essence the first step in identifying any hazards that may be associated with the food, after which the risks to human health are evaluated.

Role of Codex Alimentarius Commission (CAC) in setting food safety standards

The CAC was created in 1963 by FAO and the World Health Organization (WHO) to develop food standards, guidelines and related texts such as codes of practice under the Joint FAO/WHO Food Standards Programme. The main purposes of this programme are protection of the health of consumers and ensuring fair trade practices in the food trade, and promoting harmonization of all food standards work undertaken by international governmental and non-governmental organizations[6]. The 23rd Session of the CAC agreed to establish the *ad hoc* Intergovernmental Task Force on Foods Derived from Biotechnology (TFFBT) under the following Terms of Reference:

- to elaborate standards, guidelines or other principles, as appropriate, for foods derived from biotechnology;

[5] At the same time, the Codex Alimentarius Commission also published a third document, *Guideline for the Conduct of Food Safety Assessment of Foods Produced Using Recombinant-DNA Microorganisms.*

[6] http://www.codexalimentarius.net/web/index_en.jsp

- to coordinate and closely collaborate, as necessary, with appropriate Codex Committees within their mandate as related to foods derived from biotechnology;
- to take full account of existing work carried out by national authorities, FAO, WHO, other international organizations and other relevant international fora.

The Task Force successfully completed its work within the original four-year time frame, culminating with the publication of the Codex Principles and Guideline.

Table 2.1. Some key international consultations addressing the safety assessment of foods derived from recombinant-DNA plants (1990-2006)

Year	*Organization*	*Title and link (where available)*
1990	FAO/WHO	Strategies for assessing the safety of foods produced by biotechnology, a joint FAO/WHO consultation. Geneva, Switzerland, 5–10 Nov. 1990. (http://www.who.int/foodsafety/publications/biotech/1990/en/index.html)
1990	IFBC	Biotechnologies and food: assuring the safety of foods produced by genetic modification. Regulatory Toxicology and Pharmacology, 12: S1–S196.
1993	WHO	Health aspects of marker genes in genetically modified plants. Report of a WHO Workshop. Copenhagen, Denmark, 21–24 Sept. 1993.
1994	WHO	Application of the principles of substantial equivalence to the safety evaluation of foods or food components from plants derived by modern biotechnology. Report of a WHO Workshop, Copenhagen, Denmark, 31 Oct.–4 Nov. 1994.
1996	FAO/WHO	Biotechnology and food safety. Report of a Joint FAO/WHO Consultation, Rome, Italy, 30 Sept.–4 Oct. 1996. FAO Food and Nutrition Paper No. 61.
1996	ILSI	ILSI Allergy and Immunology Institute (AII) guidance for assessing the allergenic potential of foods derived from biotechnology.
1997	OECD	Safety assessment of new foods: results of an OECD survey of serum banks for allergenicity testing, and use of databases. (http://www.olis.oecd.org/olis/1997doc.nsf/LinkTo/sg-icgb(97)1-final)
1998	OECD	Report of the OECD workshop on the toxicological and nutritional testing of novel foods. (http://www.olis.oecd.org/olis/1998doc.nsf/LinkTo/sg-icgb(98)1-final)
2000	FAO/WHO	Report of a Joint FAO/WHO Expert Consultation on foods derived from biotechnology – safety aspects of genetically modified foods of plant origin. WHO Headquarters, Geneva, Switzerland, 29 May–2 June 2000. (http://www.fao.org/ag/agn/agns/biotechnology_expert_2000_en.asp)
2000	CAC	First session of the Codex ad hoc Intergovernmental Task Force on Foods Derived from Biotechnology. Chiba, Japan, Mar. 2000. (http://www.who.int/foodsafety/publications/biotech/ctf_march2000/en/index.html)
2001	FAO/WHO	Allergenicity of genetically modified foods, a joint FAO/WHO consultation on foods derived from biotechnology. Rome, Italy, 22–25 January 2001.
2001	CAC	Second session of the Codex ad hoc Intergovernmental Task Force on Foods Derived from Biotechnology. Chiba, Japan, Mar. 2001. (http://www.who.int/foodsafety/publications/biotech/ctf_march2001/en/index.html)
2002	OECD	Report of the OECD Workshop on the nutritional assessment of novel foods and feeds. (http://www.olis.oecd.org/olis/2002doc.nsf/LinkTo/env-jm-mono(2002)6)
2002	CAC	Third session of the Codex *ad hoc* Intergovernmental Task Force on Foods Derived from Biotechnology. Yokohama, Japan, March 2002. (http://www.who.int/foodsafety/publications/biotech/ctf_march2002/en/index.html)
2002	WHO	The stakeholders' meeting on WHO draft document "WHO – modern food biotechnology, human health and development: an evidence-based study". WHO, Geneva.
2003	CAC	Fourth session of the Codex ad hoc Intergovernmental Task Force on Foods Derived from Biotechnology. Yokohama, Japan, March 2003. (http://www.who.int/foodsafety/publications/biotech/july2003/en/index.html)
2003	OECD	Report on the questionnaire on biomarkers, research on the safety of novel foods and feasibility of post-market monitoring. (http://www.olis.oecd.org/olis/2003doc.nsf/LinkTo/env-jm-mono(2003)9)
2006	FAO	FAO expert consultation on biosafety within a biosecurity framework: Contributing to sustainable agriculture and food production. 28 February–3 March 2006, Rome, Italy. (http://www.fao.org/ag/agn/agns/meetings_consultations_2006_en.asp)

List of international consultations on food safety

Several international organizations have identified the need to convene experts in order to address the scientific and other issues raised regarding the safety aspects of foods derived from recombinant-DNA plants or the consequence of their release into the environment, to rationalize the large number of discussions taking place on the topic in different countries to which these products are being targeted. Organizations such as FAO, WHO, OECD, ILSI and IFBC played an important role in the 1990s by facilitating and supporting several expert consultations on the subject, which were followed by the establishment of the Codex Alimentarius Commission in 2000. The major references are listed in the Table 2.1 ●

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).

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[7]. 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.

7 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).

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).

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 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

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Additional resources

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4. The framework for the safety assessment of foods derived from recombinant-DNA plants

Introduction

Recombinant-DNA plants developed for food purposes have undergone safety assessment procedures, as required by various national regulatory systems, since the early 1990s. The frameworks used to structure the safety assessments have been continually developed by international organizations and standard-setting bodies to ensure the safety of products and to promote trade through harmonized regulations. The concept of substantial equivalence was introduced by OECD in 1993 as a feasible way of structuring the safety assessment of recombinant-DNA plants (OECD, 1993). The concept was later adopted by the WHO and FAO as a useful starting point for the safety assessment of recombinant-DNA plants, and now represents an essential component of all regulatory frameworks on a global scale. The rationale behind the concept's utility and adoption is that recombinant-DNA plants developed for food purposes are considered to be essentially equivalent (chemically) to their conventional counterparts, with the exception of the few defined changes that have been introduced.

Extensive general biological characterization and toxicological testing are not therefore thought to be necessary because the comparative approach should reveal relevant biological differences. Safety assessment of recombinant-DNA plants developed for food purposes is nevertheless often based on additional extensive data collected on the immunological and toxicological properties of the new plant variety. The current framework of safety assessment is thus based on both the structured comparative basis enshrined in the concept of substantial equivalence and additional analyses of the toxicological and immunological properties of the intentional and potential unintentional effects of the introduced genetic modifications. The goal of the safety assessment of foods derived from recombinant-DNA plants is to examine the intentional and unintentional consequences of the specific modification on the food components and to establish a comparative safety level by drawing on the history of safe use of the conventional plant counterpart.

The Codex framework of the safety assessment

Based on the Codex "Principles for the Risk Analysis of Foods Derived from Modern Biotechnology" (2003), the Codex "Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants" was introduced in 2003. This training tool provides a detailed introduction to the conduct of food safety assessment based on the Codex framework for the safety assessment of GM foods (CAC/GL45-2003). The stepwise approach to the safety assessment described in the Codex Guideline is presented with reference to Codex guideline paragraphs 18–21.

CODEX GUIDELINE PARAGRAPH 18.
The safety assessment of a food derived from a recombinant-DNA plant follows a stepwise process of addressing relevant factors that include:

A) Description of the recombinant-DNA plant;
B) Description of the host plant and its use as food;
C) Description of the donor organism(s);
D) Description of the genetic modification(s);
E) Characterization of the genetic modification(s);
F) Safety assessment:
   a) expressed substances (non-nucleic acid substances);
   b) compositional analyses of key components;
   c) evaluation of metabolites;
   d) food processing;
   e) nutritional modification; and
G) Other considerations.

CODEX GUIDELINE PARAGRAPH 19. In certain cases, the characteristics of the product may necessitate development of additional data and information to address issues that are unique to the product under review.

CODEX GUIDELINE PARAGRAPH 20. 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 at request. Data should be obtained using sound scientific methods and analysed using appropriate statistical techniques. The sensitivity of all analytical methods should be documented.

CODEX GUIDELINE PARAGRAPH 21. The goal of each safety assessment is to provide assurance, in the light of the best available scientific knowledge, that the food does not cause harm when prepared, used and/or eaten according to its intended use. 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. In essence, therefore, 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.

The specific data requirements in the Codex Guideline for describing the features of recombinant-DNA plants are outlined in paragraphs 22–33, and are explained in further detail in the following sections.

Description of the recombinant-DNA plant

A recombinant-DNA plant is produced as a result of successful gene transfer (transformation) followed by stable integration of the recombinant-DNA (transgene) into the nuclear chromosome(s) or organelle genome(s) of the plant. The biotechnologist uses classical plant breeding techniques such as selfing to make this initial plant homozygous at the recombinant locus (loci). The recombinant-DNA can then be stably transferred through generations without segregation. The name of the progeny of such a recombinant-DNA plant is also defined by and refers to the initially produced recombinant-DNA plant. Each plant lineage produced from a successful transfer, plant regeneration and propagation is called an "event" or a "case".

It is important for the safety assessor to understand the recombinant-DNA plant to be evaluated. For example, a clear understanding of the term "event" is essential to the application of a "case-by-case" safety assessment. Because each "event" represents a unique insertion site (or sites) of the recombinant-DNA (transgene), the resulting phenotypic properties of the regenerated recombinant plants are likely to differ. Thus, whereas the general biological properties of the recombinant-DNA will be similar across different insertion "events", potential unintentional effects on the host genome may vary because the insertions may cause different effects depending on their location and insertion number (see Box 4.1). An "event" may represent a plant with a single insert, or with multiple inserts transferred at the same time. For example, a single event may comprise several insertions of recombinant-DNA that encode both insecticide resistance and herbicide resistance, if these traits were transferred at the same time.

CODEX GUIDELINE PARAGRAPH 22. A description of the recombinant-DNA plant being presented for safety assessment should be provided. This description should identify the crop, the transformation event(s) to be reviewed and the type and purpose of the modification. This description should be sufficient to aid in understanding the nature of the food being submitted for safety assessment.

Plants containing recombinant-DNA from independent transfer events have "stacked" traits, and are often produced by crossing plant cultivars that each carry unique and well characterized "events". In this way, more recombinant-DNA insertions (and "events") that have been selected based on good performance in their original recipient host can be assembled in a single new plant variety. Plants with stacked recombinant-DNA insertions (transgenes) are also evaluated for potential interactions occurring between the DNA insertions, as a part of the safety assessment.

The first two to three pages of the example dossier extracts provided with this tool contain relevant descriptive information to provide the safety assessor with the key characteristics and intended purpose of the recombinant-DNA plant.

Description of the host plant and its use as food

Paragraphs 23–25 request information on the host plant and its known uses for food. A thorough knowledge of the non-modified host plant is necessary to apply the concept of substantial equivalence as a starting point for establishing safety. In the case of food safety assessment, this descriptive knowledge is critical for identifying the natural range and variation of key nutritional components, and of known toxicants (e.g. alkaloids in potatoes and tomatoes, curcurbiticin in squash and zucchini), antinutrients and potential allergens. These compounds and their respective concentrations will vary between crops, cultivars and growth conditions in a similar way to those of conventional varieties.

Natural variations in such compounds are known as and described by the "baseline level". Efforts are underway to establish databases that contain descriptive data on the range of baseline levels for key chemical compounds naturally present in crop plants. Crop plants naturally contain several thousand chemical compounds, of which many will cause undesired effects in toxicological tests if extracted singly and administered in high doses to experimental animals. It is therefore challenging to evaluate the biological effects potentially caused by minor variations or fluctuations in the levels of a particular plant compound. Therefore, knowledge of the natural variation in the baseline level of key compounds in conventional varieties of the plant is of great use in the safety assessment of complex data sets obtained from chemical analysis of recombinant-DNA plants.

Post-harvest processing of plant components may also alter the levels of particular plant compounds that are of nutritional value. Hence knowledge of the use, processing and consumption, as well as the properties, of the final product of the conventional food crop is important in establishing a sound basis for appropriate comparison with the foods derived from recombinant-DNA plants. Such information is provided in the example documents/dossiers.

An information source that provides extensive information on host plant biology is the OECD Consensus Documents. These consensus documents comprise technical information for use during the regulatory assessment of products of biotechnology. They focus on the biology of organisms (such as plants, trees or micro-organisms) or the introduced traits and can be accessed at: http://www.oecd.org/document/51/0,2340,en_2649_34385_1889395_1_1_1_1,00.html

CODEX GUIDELINE PARAGRAPH 23. A comprehensive description of the host plant should be provided. The necessary data and information should include, but need not be restricted to:

A) common or usual name; scientific name; and taxonomic classification;
B) history of cultivation and development through breeding, in particular identifying traits that may adversely impact on human health;
C) information on the host plant's genotype and phenotype relevant to its safety, including any known toxicity or allergenicity; and
D) history of safe use for consumption as food.

CODEX GUIDELINE PARAGRAPH 24. Relevant phenotypic information should be provided not only for the host plant, but also for related species and for plants that have made or may make a significant contribution to the genetic background of the host plant.

CODEX GUIDELINE PARAGRAPH 25. The history of use may include information on how the plant is typically cultivated, transported and stored, whether special processing is required to make the plant safe to eat, and the plant's normal role in the diet (e.g. which part of the plant is used as a food source, whether its consumption is important in particular subgroups of the population, what important macro- or micro-nutrients it contributes to the diet).

Description of the donor organism(s)

Information about the natural history of the donor organism for the recombinant-DNA sequences is required, particularly if the donor or other members of

CODEX GUIDELINE PARAGRAPH 26. Information should be provided on the donor organism(s) and, when appropriate, on other related species. It is particularly important to determine if the donor organism(s) or other closely related members of the family naturally exhibit characteristics of pathogenicity or toxin production, or have other traits that affect human health (e.g. presence of anti-nutrients). The description of the donor organism(s) should include:

A) its usual or common name;
B) scientific name;
C) taxonomic classification;
D) information about the natural history as concerns food safety;
E) information on naturally occurring toxins, anti-nutrients and allergens; for microorganisms, additional information on pathogenicity and the relationship to known pathogens; and
F) 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).

its genus normally exhibit characteristics of pathogenicity or toxin production, or have other traits that affect human health. If the donor organism contains known allergens particular caution must be exercised (Codex Guideline paragraph 26). When the food derived from recombinant-DNA plants contains genes from such sources, it is assumed that the novel gene product is allergenic unless proven otherwise. The assessment of allergenicity takes this aspect into account. In cases where the recombinant-DNA originates from sources with no history of allergenicity, the current approach to assessing allergenicity or toxicity relies primarily upon amino acid sequence comparisons and the stability of the novel protein to digestion and processing. Notably, this latter comparison is not made with respect to the conventional counterpart, but draws on a broad knowledge base regarding the biological properties of known allergens in food.

Currently, most commercially used DNA sequences inserted into recombinant-DNA plants are collected from commonly occurring soil bacteria and pathogenic plant bacteria and viruses, and hence they often have a known history in agriculture. Establishing prior human exposure to the recombinant-DNA source is useful as a starting point to identify possible toxic and allergenic properties of the gene products. Nevertheless, care should be taken in drawing safety inferences from such information, given the potentially altered expression levels, cellular locations and exposure routes of the recombinant-DNA derived proteins. Information is provided on the donor sources in the example documents/dossiers.

The OECD Consensus Documents also provide information on the biology of gene donors: http://www.oecd.org/document/51/0,2340,en_2649_34385_1889395_1_1_1_1,00.html

Description of the genetic modification(s)

The data requirements related to the genetic modifications serve two purposes: (i) to allow a detailed understanding of the resulting genetic insertions and their locations in the host plant; (ii) to allow unique identifiers to be constructed based on the event-specific insertion sites of the recombinant-DNA in the plant host genome (Codex Guideline paragraph 27). The latter information can be important both for the developer of a recombinant-DNA plant, as a means to ensure commercial distribution and use, and for some countries with mandatory food labelling requirements, to allow event-specific monitoring of recombinant-DNA in the food chain. For the biological safety assessment, it is important to have information on DNA insertion numbers and sites in order to evaluate the effect of the insertions on the host plant genome and to predict potential phenotypic changes. A detailed description of the molecular characteristics of the recombinant-DNA plant is required in order to demonstrate that the developer has critically analysed the plant and its products, including all introduced genes and expressed proteins. It should be noted that the recombinant-DNA plants have undergone extensive selective breeding subsequent to the initial gene transfer event and prior to seeking regulatory approval. Thus, the developer is likely to provide a range of data in the application dossier to demonstrate that the recombinant-DNA plant expresses only the intended phenotypic changes. As seen from the example documents/dossiers, extensive information on the characterization of the genetic modifications is provided.

The method by which the novel traits are introduced into the host plant determines, in part, the information required for the safety assessment of the genetic properties of the plant (Codex Guideline paragraph 28–29). The two principal methods for introducing new genetic

CODEX GUIDELINE PARAGRAPH 27. Sufficient information should be provided on the genetic modification to allow for the identification of all genetic material potentially delivered to the host plant and to provide the necessary information for the analysis of the data supporting the characterization of the DNA inserted in the plant.

CODEX GUIDELINE PARAGRAPH 28. The description of the transformation process should include:
A) information on the specific method used for the transformation (e.g. *Agrobacterium*-mediated transformation);
B) information, if applicable, on the DNA used to modify the plant (e.g. helper plasmids), including the source (e.g. plant, microbial, viral, synthetic), identity and expected function in the plant; and
C) intermediate host organisms including the organisms (e.g. bacteria) used to produce or process DNA for transformation of the host organism.

CODEX GUIDELINE PARAGRAPH 29. Information should be provided on the DNA to be introduced, including:
A) the characterization of all the genetic components including marker 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.

material into plant cells are (i) *Agrobacterium*-mediated transformation and (ii) microprojectile bombardment.

(i) *Agrobacterium*-mediated gene transfer. *Agrobacterium tumefaciens* is a soil-borne phytopathogen that naturally uses genetic transformation processes to subvert the metabolic machinery of the host plant cell. It does so to divert some of the host's organic carbon and nitrogen supplies to the production of nutrients (opines) that can be specifically catabolized by the invading bacteria. Parasitized cells are also induced to proliferate. Crown gall tumour disease is a direct result of the incorporation of a region of transfer-DNA (T-DNA) from a large (150–250 kb) circular Ti (tumour-inducing) plasmid, carried by *A. tumefaciens*, into the host plant genome. An understanding of this natural transformation process, together with the realization that any foreign DNA placed between the T-DNA border sequences can be transferred to plant cells, led to the construction of the first vector and bacterial strain systems for plant transformation (for a review see Hooykaas and Schilperoort, 1992). Since the first record of a transgenic tobacco plant expressing foreign genes, great progress has been made in understanding *Agrobacterium*-mediated gene transfer at the molecular level. *Agrobacterium tumefaciens* naturally infects only dicotyledonous plants, although methods for *Agrobacterium*-mediated gene transfer into monocotyledonous plants have now been developed for rice (Hiei *et al.*, 1994; Cheng *et al.*, 1998), banana (May *et al.*, 1995), maize (Ishida *et al.*, 1996), wheat (Cheng *et al.*, 1997) and sugarcane (Arencibia *et al.*, 1998; Enríquez-Obregón *et al.*, 1998). A thorough analysis of the strategies for practical application of this method has been published (Birch, 1997). *Agrobacterium*-mediated transformation of plant tissue generally results in a low copy number DNA insertion, small numbers of rearrangements, and higher transformation efficiency than direct DNA delivery techniques such as microprojectile bombardment (Powlowski and Somers, 1996; Gelvin, 1998).

(ii) Microprojectile bombardment-mediated gene transfer. Microprojectile bombardment (also known as microparticle bombardment and biolistic transformation) is a technique used to deliver DNA directly to the host genome, and has proven to be useful for the transformation of plant tissues recalcitrant to *Agrobacterium* infection. In short, plasmid or linearized DNA containing the gene(s) of interest is fixed to tungsten or gold particles (microcarriers), which are delivered to host cells at high speed so as to penetrate the plant cells. In the cell, the DNA may separate from the microcarrier and become integrated into the host genome. Microprojectile bombardment can be used to transform tissue explants of most plant species as long as the transformed plant tissue can be regenerated to produce whole plants. As seen from the example documents/dossiers, details on the gene transfer method used and a molecular analysis of the resulting DNA insertion are provided as a standard part of the application for regulatory approval/notification.

Box 4.1. Mechanistic aspects of the transformation process relevant to safety assessment of recombinant-DNA plants

Length and copy numbers of DNA transferred.
It was assumed until 1995 that in *Agrobacterium*-mediated gene transfer the sequences between the left and right borders of the T-DNA were the only transgenic elements transferred to the recipient host. However, Ramanathan and Veluthambi (1995), Wenck *et al.* (1997) and Kononov *et al.* (1997) all demonstrated that plasmid backbone sequences beyond the borders of the T-DNA could be integrated together with the genes of interest. Experiments by Kononov *et al.* (1997) demonstrated that plasmid backbone sequences could be integrated into the host genome coupled with either the right or left border sequences, or as an independent unit unlinked from the T-DNA. The T-DNA can also integrate into the host genome in patterns other than as a single copy at a single site. Multiple copies in direct or inverted repeats and other complex patterns may also occur. The presence of multimeric T-DNA inserts, especially inverted repeat structures, is linked to the phenomenon of transgene silencing (Gelvin, 1998).

In particle bombardment-mediated gene transfer, the transgene integration pattern varies from the full-length introduced transgene to transgene rearrangements that differ in size from the full length insert, occasional concatenation of introduced plasmids carrying the transgene, and variation in copy number among the full-length and partial transgenic elements (Powlowski and Somers, 1996). The copy number of transgene insertions varies from 1 to 20 or more, in addition to the insertion of partial transgene fragments. Multiple copies usually cosegregate as a transgenic locus, indicating that the sequences are either integrated into tightly linked loci or into a single locus, rather than randomly integrated throughout all chromosomes (Powlowski and Somers, 1996). Molecular characterization of transgenic plants produced through microparticle bombardment has provided evidence of extensive rearrangements of transgenic sequences (Powlowski and Somers, 1996). These rearrangements may be observed in Southern blot analyses as hybridizing fragments of a different size to the full-length DNA insert. Larger fragments are indicative of concatenation (head to head or head to tail)[8]. Larger than full-length fragments of transgenic DNA may also be caused by interspersion of inserted DNA with host DNA. For instance, Powlowski and Somers (1998) reported that each of thirteen transgenic oat lines transformed using microparticle bombardment had intact copies of the transgene, as well as multiple, rearranged, and/or truncated transgene fragments. The number of insertion sites varied from 2 to 12, and all fragments of the transgenic DNA cosegregated. The authors demonstrated that the transgenic DNA was interspersed with host DNA. This phenomenon has also been reported for rice (Cooley *et al.*, 1995).

Variation in gene expression levels based on insertion site.
For both gene transfer methods, plants transformed independently with the same plasmid will commonly have different levels of expression, a phenomenon that is not always correlated with copy number (Gelvin, 1998). Instead, differential expression of transgenes has been attributed by some to positional effects, in which the position of the DNA integration site in the host genome affects the level of transgene expression. However, other research has indicated that factors in addition to, or other than, the position of the site of integration also contribute to the level of transgene expression (Gelvin, 1998). This may be caused by the variable arrangements that transgene sequences may have in the host genome. Variable expression of transgenes, or gene silencing,[9] is a documented phenomenon in transgenic plants.

Due to commercial business information claims, the exact technical and practical laboratory details of the recombinant-DNA transfer protocols are rarely provided in the application dossier. Some of the general mechanistic aspects of the transformation process that are relevant to safety assessment of the generated recombinant-DNA plants are explained in more detail in Box 4.1.

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[8] Concatemers of the DNA insert may be detected by extensive Southern blot analysis involving digestion of the genomic DNA with a restriction enzyme that cuts at a single site within the transgenic element; multiple copies of the DNA insert will then be resolved by Southern blot analysis. Concatemers may be formed by homologous recombination of the transformed DNA or by blunt end ligation of cohesive ends produced by limited exonuclease activity. Smaller than full-length fragments are evidence of deletions and truncations.

[9] Gene silencing can result from interactions between multiple copies of transgenes and related endogenous genes and is associated with homology-based mechanisms that act at either the transcriptional or post-transcriptional level (Matzke and Matzke, 1998). Silencing that results from the impairment of transcription initiation is often associated with cytosine methylation and/or chromatin condensation (Fagard and Vaucheret, 2000) while post-transcriptional silencing (cosuppression) involves enhanced RNA turnover in the cytoplasm (Matzke and Matzke, 1998). A third category of silencing has also been proposed for the consequences of positional effects, in which flanking plant DNA and/or unfavourable chromosomal location exert a silencing effect on the transgene (Matzke and Matzke, 1998). According to Matzke and Matzke (1998), this type of silencing reflects the epigenetic state of host sequences flanking the insertion site or the tolerance of particular chromosome regions to insertion of foreign DNA.

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5. Characterization of the genetic modification(s)

Molecular analysis of the recombinant-DNA insert

Characterization of a recombinant-DNA plant at the molecular level is performed to provide information about the composition and integrity of the inserted DNA, the number and genomic location of the single or multiple sites of insertion, and the level of expression of the introduced protein(s) over time and in different tissues and environments.

As explained in the Section 4, the process of recombinant-DNA plant production may result in a transformed plant that contains a single insert or multiple inserts present in one or several locations in the host plant genome.

Regulatory authorities examine the information on the integrity and copy number of the inserted DNA in recombinant-DNA plants. Biotechnologists usually seek to minimize the copy number and size of the inserted DNA in recombinant-DNA plants to ease the regulatory process by producing fewer genetic changes that require assessment. However, recombinant-DNA plants containing multiple copies of the inserted DNA are not necessarily less "safe" than comparable plants containing only a single copy[10].

CODEX GUIDELINE PARAGRAPH 30. In order to provide clear understanding of the impact on the composition and safety of foods derived from recombinant-DNA plants, a comprehensive molecular and biochemical characterization of the genetic modification should be carried out.

CODEX GUIDELINE PARAGRAPH 31. Information should be provided on the DNA insertions into the plant genome; this should include:

A) the characterization and description of the inserted genetic materials;
B) the number of insertion sites;
C) the organisation of the inserted genetic material at each insertion site including copy number and sequence data of the inserted material and of the surrounding region, sufficient to identify any substances expressed as a consequence of the inserted material, or, where more appropriate, other information such as analysis of transcripts or expression products to identify any new substances that may be present in the food; and
D) identification of any open reading frames within the inserted DNA or created by the insertions with contiguous plant genomic DNA including those that could result in fusion proteins.

Knowledge of the genomic locations in which the transgene(s) have been inserted in the plant genome is necessary to assess if existing genes or regulatory sequences have been affected by the insertion, which may result in altered gene expression patterns and, hence, plant phenotype. To assess whether new protein molecules could be produced from the integration of inserted DNA, DNA sequence-based bioinformatics analyses are used to determine the presence of open reading frames (ORFs) in and around the DNA insert.

An open reading frame is a part of a gene that is transcribed to produce RNA. Bioinformatics analysis is usually focused on both the newly introduced ORFs present in the DNA insert itself and the potential presence or creation of new ORFs produced from the random insertion of DNA into existing ORFs in the plant genome.

A detailed molecular characterization of the recombinant-DNA may be able to address issues related to possible positional effects that lead to variable gene expression, multiple character changes (pleiotropic

[10] One example of an "event" containing a high transgene copy number is in a line of canola (Brassica napus; event 23-198, 23-18-17) approved by the Canadian Government, which was developed by introducing a thioesterase encoding gene from the California bay tree (Laurus nobilis) to increase levels of lauric acid (12:0) and, to a lesser extent, myristic acid (14:0). The original transformation event 23 was estimated to contain 15 copies of the gene, at five independent genetic loci, as shown by Southern blot and segregation analyses.

effects) arising from the DNA insertion, or gene silencing resulting from overexpression of the inserted DNA. However, in the absence of other empirical data, such molecular analyses are unlikely to predict unforeseen effects on the concentrations of key nutrients, antinutrients or endogenous toxins. Thus, additional compositional analyses are preformed.

Where the result of the modification is the expression of a novel protein, the plant material is characterized with respect to the biochemical composition and functionality of the new gene product(s). Several methods are used to verify and measure the expression of the introduced traits in a recombinant-DNA plant. For novel protein-derived traits, serological techniques are frequently used. Such techniques (e.g. Western immunoblotting or enzyme-linked immunosorbent assay [ELISA]) are used to identify the presence of the transgene product and to quantify its level in the sampled material. If the newly inserted trait is one that does not result in the expression of a new or modified protein[11] but, for instance, results in antisense RNA sequences, other techniques (e.g. Northern blotting) are used to measure transcript production.

CODEX GUIDELINE PARAGRAPH 32. Information should be provided on any expressed substances in the recombinant-DNA plant; this should include:

A) the gene product(s) (e.g. a protein or an untranslated RNA);
B) the gene product(s)' function;
C) the phenotypic description of the new trait(s);
D) the level and site of expression in the plant of the expressed gene product(s), and the levels of its metabolites in the plant, particularly in the edible portions; and
E) where possible, the amount of the target gene product(s) if the function of the expressed sequence(s)/gene(s) is to alter the accumulation of a specific endogenous mRNA or protein.

In addition to the direct biochemical characterization of the inserted trait, regulatory authorities usually assess studies of the recombinant-DNA plant grown under various conditions. Such studies can show that the intended trait is expressed at the desired life stage of the plant cultivar, and that expression is as expected and is stable over environments and plant generations.

The overall concentration of novel proteins expressed in recombinant-DNA plant tissues is low, often less than 0.1 percent on a dry weight basis. Biosafety studies, such as acute toxicity testing (chapter 6), that require relatively large amounts of material are often not feasible using the protein purified from plant tissue. Instead, these studies normally make use of protein purified from bacterial expression systems. In such cases, it is necessary to demonstrate the functional equivalence (in terms of physiochemical properties and biological activities) of the proteins purified from the two sources[12].

Refer to the Codex Guideline paragraph 33, for each introduced trait, the expected expression pattern and stability of inheritance is usually demonstrated using data from field trials collected over several seasons and geographical locations. The genomic stability of the insert is usually shown by Southern blotting of DNA extracted from plant material sampled over several seasons and locations. Similarly, stable expression of the inserted DNA is shown by quantification of the corresponding protein or protein activity.

CODEX GUIDELINE PARAGRAPH 33. In addition, information should be provided:

A) to demonstrate whether the arrangement of the genetic material used for insertion has been conserved or whether significant rearrangements have occurred upon integration;
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 through several generations consistent with laws of inheritance. It may be necessary to examine the inheritance of the DNA insert itself or the expression of the corresponding RNA if the phenotypic characteristics cannot be measured directly;
D) to demonstrate whether the newly expressed trait(s) are expressed as expected in the appropriate tissues in a manner and at levels that are 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 host plant has been affected by the transformation process; and
F) to confirm the identity and expression pattern of any new fusion proteins.

[11] For example the FlavrSavr™ tomato, which contains an antisense sequence corresponding to the polygalacturonase encoding gene.

[12] When equivalence is demonstrated based on serological cross-reactivity between the plant and bacterial proteins, it is important to use antisera (either polyclonal or monoclonal antibodies) that have been well characterized with respect to their specificity.

The application of modern profiling technology, such as DNA/RNA microarrays, proteomics, gas chromatography coupled to mass spectrometry (GC-MS) or liquid chromatography coupled to nuclear magnetic resonance (HPLC-NMR), has the potential to broaden the data available for the safety assessment. Sensitive profiling methods may provide indications of minor or major changes at the genome level in mRNA expression or protein production, and/or changes at the level of metabolism. These broad, non-targeted approaches, which do not require prior knowledge of hypothesized changes in levels of particular plant constituents to guide the choice of method, could be of particular interest for foods derived from recombinant-DNA plants modified through the insertion of multiple genes, such as plants with nutritional or health-promoting characteristics (see also the chapter on Evaluation of metabolites).

The utility and applicability of these non-targeted techniques for generating data for risk assessment purposes need further exploration, in particular with respect to establishing and validating the relevance to food safety of any observed changes. One of the major challenges in using these techniques is that observed differences may not be easily distinguishable from natural variations (baseline fluctuations in several thousand variables) in biochemical composition due to the properties of different varieties, the stage of plant development and the health status of the plant, and environmental influences and variations in growth conditions. Profiling methods are not yet suitable for routine risk assessment purposes because the observed variation in profiles cannot be routinely linked to specific biosafety considerations. Further description of baseline ranges, cost reduction, and development and validation of methods are needed.

Randomly generated plant transformation events

The transgene is generally integrated into the host chromosome(s) upon successful application of transformation processes such as the *Agrobacterium*-mediated or biolistics (microprojectile bombardment) methods. Some insertions occur in regions of the plant genome that are not involved in any obvious function, in which case the transgene may express the novel protein as expected without causing unintended change in other plant traits.

When the random insertion occurs in a region of the plant genome that is involved in genome regulation, transcription or protein production, the insertion may lead to unintended plant phenotypes. Each of the plants recovered after the transformation process that is carrying the integrated DNA represents a unique gene transfer "event".

Because insertion of the transgene into the host plant genome occurs randomly, a large number of transformed plants are usually produced initially, each containing single or multiple copies of the transgene. Subsequent small-scale cultivation and selection-based screening will remove unintended phenotypes possessing unwanted traits and/or multiple copy insertion "events" and preserve the most suitable phenotypes for further characterization and further rounds of selection-based breeding to obtain elite cultivars.

Transgene detection using event-specific primers

Two DNA primers (each 20–30 bases long) with nucleotide sequences complementary to the DNA inserted into the recombinant-DNA plant are generally employed in a polymerase chain reaction (PCR) to detect the presence of a transgene. If both of the PCR primers are complementary to the transgene sequence, then all plant varieties and species that carry the same transgene will show the PCR amplification product, irrespective of the location of the insertion in the plant genome. However, it is possible to distinguish among the different insertion "events" of the same transgene in the same plant cultivar by designing the primer pair appropriately.

Event specificity is based on using a primer pair of which one primer is complementary to the plant genomic region adjacent to the point of insertion of the transgene, and the other primer is complementary to a region within the transgene. These primers are known as "event-specific" primers. This primer pair will only amplify a specific insertion "event" because the process of DNA insertion into plants is effectively random. Therefore, each insertion of DNA will take place at random in the plant genome and will lead to that insertion having unique flanking regions of plant DNA.

The use of event-specific primers is necessary for identifying a particular transformation event among other events carrying the same gene in the same host variety or other varieties of the same crop species. Hence, access to sequence information for the flanking regions of the integration site of the inserted DNA is necessary so that regulatory authorities can conduct event-specific monitoring of recombinant-DNA plants. Due to the large variety of plant cultivars harbouring the same transgene, monitoring of recombinant-DNA plants is typically done in two steps. Step one, which is PCR-based, determines the presence of frequently used transgene constructs, and if this is positive, a second-step (also PCR-based) is performed, which employs event-specific primers.

For examples of the use of event-specific primers, see the validated methods published online by the European Commission's Joint Research Centre: http://gmo-crl.jrc.it/default.htm

Extent of refinement at the current level of the technology

Unintended changes can result from the random insertion of DNA sequences into the plant genome, which may cause modifications in the expression of existing genes, or activation of silent genes, possibly resulting in elevated levels of native or new toxins in the food. It is emphasized that the occurrence of unintended effects is not specific to the application of recombinant-DNA technology in plants, as it also occurs in classic plant breeding. In breeding practice, backcrossing and selection based on morphology, yield, crop quality, insect/disease resistance, etc. identify lines with unwanted characteristics that are discarded[13]. Similarly, during the development of recombinant-DNA plants, modified lines that do not meet the expected agronomic, safety and quality requirements will be discarded, resulting in the elimination of many unintended effects from the tissue culture or DNA insertion process[14].

A limitation in the current application of recombinant-DNA technology in plants is the inability to direct the insert DNA (transgene) into a specific genomic location. Further developments in the technology leading to the option to specifically target the DNA insertion to particular genomic regions may eliminate unintended effects such as positional effects on transgene expression and the influence of the insert on plant genome expression ●

[13] Reports of unintended effects that may affect human health are rare, and include examples such as low yields in barley or maize, high content of furanocoumarins in celery, and high glycoalkaloid content in potatoes.

[14] Examples of unintended effects that have been observed in recombinant-DNA plants are potatoes with abnormal tuber tissue or with reduced glycoalkaloid content, soybeans with higher lignin content, and rice with increased Vitamin B6 content or higher levels of certain carotenoid derivatives.

6 Assessment of possible toxicity of foods derived from recombinant-DNA plants

Introduction

Risk assessment also takes into consideration the estimation and assessment of the level and frequency of intake of food from recombinant-DNA plants. This takes into account how frequently and to what extent the population would be exposed to newly expressed substances such as proteins, metabolites or endogenous compounds that are at altered levels in food due to the newly inserted gene (and/or other unintended effects resulting from genetic modification).

Conventional toxicological tests adopted from those originally developed for chemicals (i.e. food additives, pesticides and food contaminants) may be an appropriate approach to determining the safety of newly expressed substances. It is possible to determine the NOEL (no observed [adverse] effect level) of the new substance and subsequently the safety factor related to the level of exposure expected in the general population. Hence the safety factor is applied to derive the acceptable or tolerable daily intake. If such studies are to be undertaken, they should be designed according to the identity and biological function of the substances under consideration.

Conventional toxicology studies on the safety of whole foods are, however, not meaningful in practice because foods are complex mixtures of compounds characterized by wide variation in composition and nutritional value. Detecting any potential adverse effects and relating these conclusively to an individual characteristic of the food can therefore be extremely difficult. These difficulties in applying conventional toxicology approaches to recombinant-DNA plants have led to the development of the concept of substantial equivalence. This conceptual approach acknowledges that the goal of the assessment is not to establish absolute safety but to consider whether foods derived from recombinant-DNA plants are as safe as their traditional counterparts or not.

Conceptual approach to toxicity studies

The conceptual approach to the assessment of potential toxic properties of food involves biochemical characterization of the novel product from the inserted DNA element by *in vitro* digestibility studies, determination of the amino acid sequence similarity to known toxins, and acute oral toxicity studies based on an animal model. If on the basis of these studies a longer-term effect can be assumed then additional subchronic and chronic toxicity testing will be required. The *in vitro* digestibility studies are performed to determine the resistance of the novel product to acid, thus simulating the conditions in gastric and intestinal fluids. The sequence of the six amino terminal amino acids is compared with the amino terminal of the amino acid sequence of known toxins to determine their similarity. If the similarity is significant, it is possible that the novel product from the inserted gene is a toxin. The novel product is then subjected to subchronic toxicological studies to determine the safety factor for consumption relative to the exposure of the general population.

CODEX GUIDELINE PARAGRAPH 34. *In vitro* nucleic acid techniques enable the introduction of DNA that can result in the synthesis of new substances in plants. The new substances can be conventional components of plant foods such as proteins, fats, carbohydrates or vitamins which are novel in the context of that recombinant-DNA plant. New substances might also include new metabolites resulting from the activity of enzymes generated by the expression of the introduced DNA.

CODEX GUIDELINE PARAGRAPH 35. The safety assessment should take into account the chemical nature and function of the newly expressed substance and identify the concentration of the substance in the edible parts of the recombinant-DNA plant, including variations and mean values. Current dietary exposure and possible effects on population sub-groups should also be considered.

CODEX GUIDELINE PARAGRAPH 36. 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 plants that do not normally express those toxic or anti-nutritious characteristics. This assurance is particularly important in cases where a recombinant-DNA plant is processed differently from a donor plant, since conventional food processing techniques associated with the donor organisms may deactivate, degrade or eliminate anti-nutrients or toxicants.

CODEX GUIDELINE PARAGRAPH 37. For the reasons described in Section 3, 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.

CODEX GUIDELINE PARAGRAPH 38. 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, lectins) as well as stability to heat or processing and to degradation in appropriate representative gastric and intestinal model systems. Appropriate oral toxicity studies[15] may need to be carried out in cases where the protein present in the food is not similar to proteins that have previously been consumed safely in food, and taking into account its biological function in the plant where known.

CODEX GUIDELINE PARAGRAPH 39. Potential toxicity of non-protein substances that have not been safely consumed in food should be assessed on a case-by-case basis depending on the identity and biological function in the plant of the substance and dietary exposure. The type of studies to be performed may include studies on metabolism, toxicokinetics, sub-chronic toxicity, chronic toxicity/carcinogenicity, reproduction and development toxicity according to the traditional toxicological approach.

CODEX GUIDELINE PARAGRAPH 40. This may require the isolation of the new substance from the recombinant-DNA plant, or the synthesis or production of the substance from an alternative source, in which case the material should be shown to be biochemically, structurally, and functionally equivalent to that produced in the recombinant-DNA plant.

[15] Guidelines for oral toxicity studies have been developed in international fora, for example, the OECD Guidelines for the Testing of Chemicals.

The conceptual approach to evaluating the toxicity of an introduced substance is described in Codex Guideline paragraphs 34–40.

Methods used to determine absence of toxicity

The requirements for and methods used to determine whether the new substance from the inserted gene is a toxin or not are described in the Codex Guideline paragraphs 34–4. Large amounts of purified protein expressed by the transgene are required for toxicity studies. The levels obtainable in plant tissue are generally not sufficient, and the proteins are therefore usually extracted from GM micro-organisms (such as *Escherichia coli*) engineered to express the protein in large amounts. In such cases, biochemical and functional equivalence of the bacterially derived version and the plant-expressed version must be demonstrated.

Animal feeding studies are usually performed to establish the absence of acute and subchronic toxicity. Animal feeding studies nevertheless have recognized limitations. It is important to realize that whereas carefully performed animal feeding studies demonstrating a lack of effect on selected physiological outcomes can be useful, the studies do not provide complete assurance of safety, because of the usual caveats with extrapolating results from other animals to humans. The results should be considered as “confirmatory” and “safety assuring”

CODEX GUIDELINE PARAGRAPH 10. The use of animal models for assessing toxicological endpoints 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.

CODEX GUIDELINE PARAGRAPH 11. Animal studies cannot readily be applied to testing the risks associated with whole foods, which are complex mixtures of compounds, 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 which 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 foods. 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.

CODEX GUIDELINE PARAGRAPH 12. 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 derived from food plants, including recombinant-DNA plants. This has been addressed by the development of a multidisciplinary approach for assessing safety which takes into account both intended and unintended changes that may occur in the plant or in the foods derived from it, using the concept of substantial equivalence.

and are an additional component of the overall safety assessment in those circumstances in which they are warranted. The advantages and limitations of animal studies that must be taken into consideration in the determination of the safety of the foods derived from recombinant-DNA plants are discussed in the Codex Guideline paragraphs 10–12.

Feeding studies that use whole foods rather than isolated compounds may be appropriate when there are significant compositional changes in the food derived from recombinant-DNA plants; see Codex Guideline paragraph 53.

The ethical aspects of and necessity for animal feeding studies are issues that must be continually reconsidered to avoid unnecessary animal suffering. The Joint FAO/WHO Expert Consultation on Foods Derived from Biotechnology in 2000 (*Safety aspects of genetically modified foods of plant origin,* Section 4.2, paragraph 4.2.2) provided a useful discussion of the need for animal studies (Box 6.1).

It is generally considered that a subchronic study in rodents of 90 days' duration is the minimum requirement to demonstrate the safety of repeated consumption of foods derived from recombinant-DNA plants in the diet. The Joint FAO/WHO Expert Consultation on Foods Derived from Biotechnology in 2000 (*Safety testing of food additives and contaminants and the long-term evaluation of foods produced by biotechnology*, page 4) provided a useful discussion of subchronic toxicity studies (summarized in Box 6.2).

The document produced by the United States Food and Drug Administration on the toxicological principles of the safety assessment of food ingredients (US FDA, 2003)

CODEX GUIDELINE PARAGRAPH 53. Some foods may require additional testing. For example, animal feeding studies may be warranted for foods derived from recombinant-DNA plants 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 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.

Box 6.1. Need for animal studies (FAO/WHO, 2000)

If the characterization of the food indicates that the available data are insufficient for a thorough safety assessment, animal testing may be deemed necessary. This would particularly be the case if the food were expected to make a significant dietary contribution, if there is no history of consumption of the novel gene product or if the modification affects several metabolic pathways.

In the situation where the genetically modified food differs from the traditional counterpart by the presence of one or a few new genes and their products, it may be possible to isolate and study these in a manner analogous to conventional toxicity testing of food additives.

However it is essential to ensure that the material tested is biochemically and functionally equivalent to that produced in the genetically modified food. This provides the possibility of increasing the sensitivity of toxicity tests compared with that possible if the products of the genetically modified plants had been fed directly and avoids some of the artefacts that can occur in toxicity tests on whole foods. However, this strategy is only applicable if the preceding detailed analysis does not reveal significant changes other than those expected. Otherwise testing of the whole food may be required. When animal testing is conducted on the whole food, it should generally be on the food as consumed by humans. The type of animal study would need to be considered on a case by case basis. In addition to investigating potential toxicological effects, animal studies may also be required if the genetic modification directly or indirectly affects the content or bioavailability of nutrients.

Where toxicological studies are considered necessary to assess the safety of long term consumption of a food in the diet, it is generally considered that a sub-chronic study of 90-days duration is the minimum requirement to demonstrate the safety of repeated consumption of a food in the diet. This may need to be preceded by a pilot study of short duration to ensure that the diet is palatable to the test species and that the levels of incorporation of the test article are appropriate, e.g. the control diet containing the equivalent level of the comparator does not produce effects, as a result of normal levels of natural toxicants present in traditional foods accepted as safe. The highest dose level used in any animal study should be the maximum achievable without causing nutritional imbalance while the lowest level used should be comparable to the anticipated human intake.

The need for additional toxicological tests should be considered on a case-by-case basis taking into account the results of the 90-day study and other studies. For example, proliferative changes in tissues during the 90-day study may indicate the need for a longer-term toxicity study.

Conventional toxicological tests are of limited value in assessing whole foods, including genetically modified foods. Based on the maximum levels of the whole food that can be incorporated into experimental diets as indicated previously, a margin of safety may be estimated based on the absence or nature of adverse effects and likely human exposure. Improved experimental designs should take into account the need for nutritionally adequate animal diets, avoiding some of the inappropriate testing of foods or products.

It has been suggested that the use of biomarkers of early effects might increase diagnostic value and sensitivity of toxicity tests on foods (Schilter *et al.*, 1996). However, it will be necessary not to confuse adaptive and toxic effects in applying this approach.

Box 6.2. Toxicological studies on foods produced by biotechnology (FAO/WHO, 2000)

When a food product of biotechnology differs from a traditional food in a few well defined characteristics, these may serve to focus the safety evaluation process and determine the tests required. The toxicological focus will be on the few well defined characteristics. It may be possible to isolate and study differences in one or a few new genes and their products in a manner analogous to conventional toxicity testing of food additives.
The conventional toxicity testing of these new genes and their products is usually the standard 14-day subacute study (OECD, 1995: Guideline 407).
A substance to be tested for toxicity is usually fed to rats in a standard 14-day subacute study at a level that would reflect a very large margin of safety. The NOEL would represent the maximum level that can be incorporated into experimental diets with no adverse effects, and this could be translated to the safety factor for human exposure to the product.
Human studies should contribute to the evaluation process, and might be conducted when the i*n vivo* animal studies demonstrate no unexpected or irreversible effects[16].

A tiered approach to such studies should be adopted to investigate tolerance up to maximum levels of potential intake. The purpose is to have some confirmatory controlled clinical studies before getting into the greater complexities of general release. It is desirable that human studies are conducted as soon as possible within ethical constraints in order better to target animal studies and to avoid extensive but irrelevant animal studies. Observations from animal and human studies may reveal that the food is safe for its intended use, or may reveal unexpected indications that require more detailed investigation to confirm food safety.

[16] Joint FAO/WHO Expert Consultation on Foods Derived from Biotechnology, Topic 6: Safety testing of food additives and contaminants and the long term evaluation of foods produced by biotechnology. 29 May–2 June 2000.

Box 6.3. Technical aspects of subchronic toxicity studies (FDA, 2003)*

Subchronic toxicity studies with rodents are generally conducted for between 90 days (3 months) and 12 months. Subchronic toxicity studies are generally used to help predict appropriate doses of the test substance for future chronic toxicity studies, to determine NOELs for some toxicology endpoints or to allow future long-term toxicity studies in rodents and non-rodents to be designed with special emphasis on identified target organs. They cannot be used to determine the carcinogenic potential of a test substance.

It is essential that all non-clinical laboratory studies are conducted according to the internationally recognized guidelines[17] and good laboratory practice (GLP)[18] regulations. Other factors that must be taken into consideration are discussed below.

Test animals

The care, maintenance and housing of laboratory animals must follow the guidelines in the *Guide for the care and use of laboratory animals*[19].

The selection of species, strains and sex must take into consideration of test animals' general sensitivity. The responsiveness of particular organs and tissues of the test animals to the toxic substance to be tested must be considered when selecting rodent species, strains and substrains for toxicity studies. The selection of inbred, outbred or hybrid rodent strains for toxicity studies should be based upon the scientific questions to be answered. Moreover, the test animals should come from well characterized and healthy colonies, because recent information has suggested problems with the survivability of some strains of rats and test animals should be selected to achieve the recommended duration of the study.

The age of the test animals may result in variation in results. Testing should be conducted on young animals, and dosing should be commenced immediately after weaning, following an acclimation period of at least 5 days, and for rodents no later than 6–8 weeks of age.

An equal number of males and females of each species and strain should be used for the study. For subchronic toxicity studies, experimental and control groups should contain at least 20 rodents of each sex per group. These recommendations will help ensure that the number of animals that survive until the end of the study will be sufficient to permit a meaningful evaluation of toxicological effects.

The animals should be housed one per cage in order to address the following concerns.

If more than one animal is present in a cage, the feed efficiency (the relationship between feed consumed and body weight gained) cannot be determined with accuracy.

It is impossible to determine whether a decrease in body weight is due to decreased palatability or substance-mediated toxicity.

The organs and tissues from moribund and dead animals may be lost as a result of cannibalism if they are not individually caged.

The diet provided to the animals must be isocaloric and contain the same levels of nutrients (e.g. fibre and micronutrients) in both the treated and the control groups[20]. Inadequately controlled dietary variables may result in nutritional imbalances or caloric deprivation that could confound interpretation of the results of the toxicity study and alter the outcome and reproducibility of the studies.

The animals should be assigned to control and compound-treated groups in a stratified random manner; this will minimize bias and assure comparability of pertinent variables across treated and control groups (for example, mean body weight and body weight ranges). If other characteristics are to be used as the basis for randomization then that characterization should be described and justified. Animals in all groups should be placed in the study on the same day; if this is not possible because of the large number of animals in a study, animals may be placed in the study over several days. If recruitment to the study over several days is selected, a preselected portion of the control and experimental animals should be placed in the study on each day in order to maintain concurrence.

Experimental design

The animals should be exposed to the test substance on 7 days per week for a minimum of 90 consecutive days (3 months).

The route of administration of the test substance should be appropriate to the normal human exposure. A justification must be provided if alternative routes are used. Possible administration routes are described below.

The substance should be administered in the diet if the human exposure is likely to be through consumption of solid foods or a combination of solid and liquid foods. The animals should not be allowed to consume selectively either the basal diet or the test substance in the diet. Care must be taken to ensure that processes used during pelleting, such as heating, do not affect the test substance.

The test substance may be administered by dissolving in the drinking water. Alternatively, the test substance may be administered by encapsulation or oral intubation (gavage) if the human exposure is expected to be through daily ingestion of a single large dose instead of continual ingestion of small doses. Administration by gavage should be performed at approximately the same time each day, and the maximum volume of solution to be given by gavage in one dose should depend on the size of the test animal. In rodents, the volume should not exceed 1 ml/100 g body weight and for oily substances it should not exceed 0.4 ml/100g body weight. If the administered amount is to be divided into smaller doses, all must be administered within a 6-hour period.

Dose groups

At least three dose levels of the test substance should be used per sex (one dose level per group); however, ideally, four or five dose levels of the test substance should be used. A concurrent control group should be included. The appropriate dose levels for subchronic toxicity studies can be determined based on the information from acute and short-term toxicity studies.

(Continued)

17 OECD Guideline for the testing of chemicals, repeated dose 90-day oral toxicity study in rodents, 407, Sept. 1998.

18 OECD Principles of Good Laboratory Practice Directive 87/18/EEC, Directive 88/320/EEC.

19 National Research Council Institute of Laboratory Animal Resources. 1996. Guide for the care and use of laboratory animals. Washington, DC, National Academy Press.

20 Nutrient requirements of laboratory animals, 4th Revised Edition, Subcommittee on Laboratory Animal Nutrition, Committee on Animal Nutrition, Board on Agriculture, National Research Council, 1995.

Box 6.3 (cont.)

Selection of treatment doses
A minimum of three dose levels of the test substance and a concurrent control group should be used in toxicity studies. The three dose levels administered should follow the guidelines as follows:

- the high dose should be sufficiently high to induce a toxic response in the test animals;
- the intermediate dose should be sufficiently high to elicit minimal toxic effects in the test animals, such as alterations in enzyme levels or a slight decrease in body weight gain;
- the low dose should not induce toxic responses in the test animals.

Controls
A concurrent control group of test animals is required. The control group in dietary studies should be fed the basal diet.

The carrier or vehicle for the test substance should be given to control animals at a volume equal to the maximum volume of carrier or vehicle given to any dosed group of animals. Information on the toxicity of the carrier or vehicle should be available to ensure that it will not compromise the results of the study.

Observations and clinical tests: observations of test animals
Observations should be made of all animals at least once or twice a day throughout the study for general signs of pharmacological and toxicological effects, morbidity and mortality. The usual interval between observations should be at least 6 hours. Individual records should be maintained for each animal and the time of onset and characteristics and progression of any effects should be recorded, preferably using a scoring system. The clinical evaluations should not only assess the general pharmacological and toxicological effects but also neurological disorders, behavioural changes, autonomic dysfunction, and other signs of nervous system toxicity. The signs noted should include, but not be limited to, changes in skin, fur, eyes, mucous membranes, occurrence of secretions and excretions and other evidence of autonomic activity. In addition, changes in posture and response to handling, as well as the presence of clonic or tonic seizures, stereotypes or bizarre behaviour should be recorded. The development of tumours should be recorded, particularly in long-term studies. During the course of a study, toxic and pharmacological signs may suggest the need for additional clinical tests or expanded post-mortem examinations.

Body weight and feed intake data
Test animals should be weighed at least once a week. Feed consumption (or water consumption if the test substance is administered in the drinking water) should be measured every week during a subchronic toxicity study.

Clinical testing
The following tests should be performed: ophthalmological examination, haematology profiles, clinical chemistry tests, urinalyses, neurotoxicity screening/testing and immunotoxicity studies.

Necropy and microscopic examination
All test animals should be subjected to the following examinations: gross necropsy, measurement of organ weight, preparation of tissues for microscopic examination, microscopic evaluation, and histopathology of lymphoid organs.

***Reference:* US FDA.** 2003. *Toxicological principles for the safety assessment of food ingredients: Red Book 2000, November 2003. IV.C.4a. Subchronic toxicity studies with rodents.* Food and Drug Administration, Center for Food Safety and Applied Nutrition, Department of Health and Human Services.

may also be a useful source for the technical aspects of subchronic toxicity studies (summarized in Box 6.3).

Chronic toxicity studies

Chronic toxicity studies involve long-term administration of the test substance, usually in the diet or drinking water, and sometimes by gavage. Chronic toxicity studies are designed to detect possible cumulative effects on target organ(s) in a dose–response dependent manner. The need for long-term chronic toxicity studies should be addressed on a case-by-case basis, and only when the results of the 90-day or other feeding studies indicate the need to consider toxicity from a longer term-perspective.

Quality assurance

It is very important that the organizational process and the conditions under which laboratory studies are planned, performed, monitored, recorded and reported are conducted according to the principles of good laboratory practice (GLP)[18]. The principles of GLP must be applied to

[18] See page 28.

testing of chemicals to generate data on their properties and/or their safety for human health or the environment. In toxicology studies, it is essential to be certain that the data used to estimate safety are of a quality that is acceptable to all parties. It is also important in toxicology studies to establish the relationship between the changes in physiological parameters measured and the dose levels of the tested compound to which animals are exposed. Hence, good quality data are of the utmost importance and lead to accurate interpretation of the toxicity and estimation of the NOEL of the tested compound. From this interpretation, the safety factor can be established by estimating the maximum levels to which the human population can be exposed without observed adverse effects on health. Moreover, any observed differences between treated and untreated animals in the physiological parameters measured in animal experiments must be analysed statistically to establish the confidence limits of these differences.

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7 Assessment of possible allergenicity (Proteins) in foods derived from recombinant-DNA plants

Food allergies

Food allergies are adverse reactions to an otherwise harmless food or food component and involve an abnormal response of the body's immune system to specific protein(s) in foods known as "allergens". True food allergies may involve several types of immunological response (Sampson and Burks, 1996).

The most common types of food allergies are mediated by allergen-specific immunoglobulin E (IgE) antibodies[21]. IgE-mediated reactions are known as immediate hypersensitivity reactions because symptoms occur within minutes to a few hours after ingestion of the offending food. IgE-mediated reactions can occur to pollens, mould spores, animal danders, insect venoms and other environmental stimuli as well as foods. IgE-mediated reactions affect perhaps 10 to 25 percent of the population in developed countries (Mekori, 1996).

Food allergies represent a small fraction of all allergic diseases, affecting less than 2.5 percent of the population in developed countries (Anderson, 1996). Infants and young children are more commonly affected by IgE-mediated food allergies than adults; the prevalence among infants under the age of 3 years may be as high as 5 to 8 percent (Bock, 1987; Sampson, 1990).

True food allergies also include cell-mediated reactions, which involve sensitized tissue-bound lymphocytes rather than antibodies (Sampson, 1990). In cell-mediated reactions, the onset of symptoms occurs more than 8 hours after ingestion of the offending food. The role of foods in cell-mediated reactions remains uncertain (Burks and Sampson, 1993) but coeliac disease[22], also known as gluten-sensitive enteropathy, affects one in every 300 to 3 000 individuals in the population, depending upon the specific geographical region. Both IgE-mediated food allergies and gluten-sensitive enteropathy are treated with specific avoidance diets. Because in both cases the threshold dose is quite low, great care must be taken in the construction of safe and effective avoidance diets.

The Codex Alimentarius Commission has produced a list of the most common allergenic foods associated with IgE-mediated reactions on a worldwide basis, which includes peanuts, soybeans, milk, eggs, fish, crustacea, wheat and tree nuts. These commonly allergenic foods account for over 90 percent of all moderate to severe allergic reactions to foods, although an extensive literature search has revealed more than 160 foods associated with sporadic allergic reactions (Hefle *et al.*, 1996).

Allergic reactions to fresh fruits and vegetables, comprising the so-called oral allergy syndrome, are also rather common (Parker *et al.*, 1990), but these foods are not included on the Codex Alimentarius Commission list because the symptoms are typically mild and confined to the oropharyngeal region, and the allergens are unstable to heating and digestion. The list established by the Codex Alimentarius Commission also includes gluten-containing cereals (wheat, rye, barley, oats and spelt) that are implicated in the aetiology of gluten-sensitive enteropathy. Table 7.1 provides a summary of protein sequences of food allergens from foods of plant origin and their accession numbers for retrieving the sequence data from the relevant databases.

[21] IgE, or immunoglobulin E, is a protein antibody that recognizes an allergen. IgE circulates in the blood and becomes fixed on the surface of specific cells (basophils and mast cells). When IgE on the cell surface binds to an allergen, this triggers the release of chemical mediators that provoke the symptoms associated with allergic reactions.

[22] Gluten-sensitive enteropathy is a malabsorption syndrome characterized by body wasting, anaemia, diarrhoea and bone pain, along with other symptoms.

Almost all food allergens are proteins, although it is possible that other food components may act as haptens[23]. Similarly, prolamin proteins from wheat, rye, barley, etc. are involved in the elicitation of gluten-sensitive enteropathy. While the crops from which staple foods are derived contain tens of thousands of different proteins, relatively few are allergenic. The distribution of these proteins varies throughout the plant and can be influenced by environmental factors, such as climate and disease stress. Conventional breeding removes diversity from or introduces protein diversity into the food supply, but has had little, if any, effect on the allergenic potential of our major foods.

Table 7.1. Food allergen protein sequences of plant origin[1]

Species	*Common name*	*Allergen*	*Synonym/function*	*Accession[2]*
Arachis hypogea	Peanuts	*Ara* h 1	Clone P41b	L34402
			Clone 5A1	L33402
			Clone P17	L38853
		Peanut lectin	Agglutinin	S14765
Bertholletia exceisa	Brazil nut	*Ber* e 1	2S albumin (BE2S1 gene)	X54490
Brassica juncea	Leaf mustard	*Bra* j IE-L	2S albumin large chain	S35592
		Bra j IE-S	2S albumin small chain	S35591
Carica papaya	Papaya	Papain		M15203
Glycine max	Soybean	Glycinin	A1aBx subunit	X02985
			A2B1a subunit	Y00398
			A3B4 subunit	M10962
			G1 subunit	X15121
			G2 subunit	X15122
			G3 subunit	X15123
		beta-Conglycinin	alpha-subunit	X17698
			CG4 subunit	S44893
		Soy lectin	Soy agglutinin	K00821
		Kuntz trypsin inhibitor	KTi-s subtype	X80039
			KTi-a subtype	X64447
			KTi-b subtype	X64448
Hordeum vulgare	Barley	*Hor* v 1	alpha-amylase/trypsin inhibitor	S26197
		Hor v 1	alpha-amylase/trypsin inhibitor	P32360
Malus domestica	Apple	*Mal* d 1	Profilin	X83672
Oryza sativa	Rice	RAP	Rice allergenic protein	X66257
		RAG1	Rice allergen 1	D11433
		RAG2	Rice allergen 2	D11434
		RAG5	Rice allergen 3	D11430
		RAG14	Rice allergen 14	D11432
		RAG17	Rice allergen 17	D11431
Phaseolus vulgaris	Kidney bean	PR-1	Pathogenesis related protein 1	S11929
		PR-2	Pathogenesis related protein 2	S11930
Sinapis alba	White mustard	*Sin* a 1.1	2S albumin/amylase inhibitor	S54101
		Sin a 1.2	2S albumin/amylase inhibitor	PC1247
Triticum aestivum		WGA	Wheat germ agglutinin A	M25536
		WGA	Wheat germ agglutinin D	M25537
Triticum durum	Pasta wheat	WGA	Wheat germ agglutinin	J02961
Triticum turgidum	Poulard wheat	16K allergen	alpha-amylase inhibitor	S19296

[1] *Adapted from Metcalfe* et al. *(1996).*

[2] *Public domain databases: GenBank/EM BL/Genpept ver 86.0, SWISSPROT ver 30, PIR ver 41.*

[23] Haptens are small molecules that may interact with body proteins or food proteins and cause these proteins to become allergenic.

Allergenicity potential of foods derived from recombinant-DNA plants

Potential allergenicity is a concern with proteins introduced into the human diet through food derived from recombinant-DNA plants, especially when there is no history of their consumption, where the source cannot be readily identified, or when they are recombined versions of proteins from different sources. The current allergenicity assessment approach is presented in the Annex "Assessment of possible allergenicity" of the Codex Guideline (see Appendix 2). As there is no definitive test that can be relied upon to predict allergic responses in humans to a newly expressed protein, the Codex recommends that an integrated, stepwise, case-by-case approach be used in the assessment of possible allergenicity of newly expressed proteins. This approach takes into account the evidence derived from several types of information and data because no single criterion is sufficiently predictive.

In addition to the Annex, the Codex Guideline outlines approaches to allergenicity assessment in paragraphs 41–43.

Allergenicity assessment strategy

The initial steps in assessing the possible allergenicity of any newly expressed protein are the determination of the source of the introduced protein, any significant similarity between the amino acid sequence of the protein and that of known allergens, and its structural properties, including, but not limited to, its susceptibility to enzymatic degradation, heat and/or acid and enzymatic treatment.

As there is no single test that can predict the likely human IgE response to oral exposure, the first step in the characterization of newly expressed proteins should be the comparison of the amino acid sequence and certain physicochemical characteristics of the newly expressed protein with those of established allergens using a weight of evidence approach (see Box 7.1 for an outline of some important parameters used). This will require the isolation of any newly expressed proteins from the recombinant-DNA plant 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 plant. Particular attention should be paid to the choice of the expression host, because the post-translational modifications allowed by different hosts (i.e. eukaryotic vs prokaryotic systems) may have an impact on the allergenic potential of the protein.

It is important to establish whether the source is known to cause allergic reactions. Genes derived from known allergenic sources should be assumed to encode an allergen unless scientific evidence demonstrates otherwise.

CODEX GUIDELINE PARAGRAPH 41. When the protein(s) resulting from the inserted gene is present in the food, it should be assessed for potential allergenicity in all cases. An integrated, stepwise, case-by-case approach used in the assessment of the potential allergenicity of the newly-expressed protein(s) should rely upon various criteria used in combination (since no single criterion is sufficiently predictive on either allergenicity or non-allergenicity). As noted in paragraph 20, the data should be obtained using sound scientific methods. A detailed presentation of issues to be considered can be found in the Annex to this document.

CODEX GUIDELINE PARAGRAPH 42. The newly expressed proteins in foods derived from recombinant-DNA plants should be evaluated for any possible role in the elicitation of gluten-sensitive enteropathy, if the introduced genetic material is obtained from wheat, rye, barley, oats, or related cereal grains.

CODEX GUIDELINE PARAGRAPH 43. The transfer of genes from commonly allergenic foods and from foods 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.

Box 7.1. Important parameters used in the assessment of allergenicity

Source of the protein

As part of the database supporting the safety of foods derived from recombinant-DNA plants, any reports of allergenicity associated with the donor organism should be described. Allergenic sources of genes are defined as those organisms for which reasonable evidence of IgE-mediated oral, respiratory or contact allergy is available. Knowledge of the source of the introduced protein allows the identification of tools and relevant data to be considered in the allergenicity assessment. These include the availability of sera for screening purposes, documentation of the type, severity and frequency of allergic reactions, the structural characteristics and amino acid sequence of the protein, and the physiochemical and immunological properties (if available) of known allergenic proteins from that source.

Amino acid sequence homology

The purpose of a sequence homology comparison is to assess the extent to which a newly expressed protein is similar in structure to a known allergen. This information may suggest whether the protein has allergenic potential. Sequence homology searches should be performed to compare the structure of all newly expressed proteins with all known allergens. Searches should be conducted using various algorithms such as FASTA or BLASTP[24] to predict overall structural similarities. Strategies such as stepwise contiguous identical amino acid segment searches may also be performed to identify sequences that may represent linear epitopes. The size of the contiguous amino acid search should be based on a scientifically justified rationale in order to minimize the potential for false negative or false positive results[25]. Validated search and evaluation procedures should be used in order to produce biologically meaningful results.

IgE cross-reactivity between the newly expressed protein and a known allergen should be considered a possibility when there is more than 35 percent identity in a segment of 80 or more amino acids (FAO/WHO, 2001), or when other scientifically justified criteria are met. All the information resulting from the sequence homology comparison between the newly expressed protein and known allergens should be reported to allow a case-by-case scientifically based evaluation.

Sequence homology searches have certain limitations. In particular, comparisons are limited to the sequences of known allergens in publicly available databases and the scientific literature. There are also limitations in the ability of such comparisons to detect non-contiguous epitopes capable of binding specifically with IgE antibodies.

A negative sequence homology result indicates that a newly expressed protein is not a known allergen and is unlikely to be cross-reactive with known allergens. A result indicating the absence of significant sequence homology should be considered along with the other data outlined under this strategy in assessing the allergenic potential of newly expressed proteins. Further studies should be conducted as appropriate (see also Specific serum screening, below). A finding of positive sequence homology indicates that the newly expressed protein is likely to be allergenic. If the product is to be considered further, it should be assessed using serum from individuals sensitized to the identified allergenic source.

Pepsin resistance

Resistance to pepsin digestion has been observed in several food allergens; thus a correlation exists between resistance to digestion by pepsin and allergenic potential[26]. Therefore, the resistance of a protein to degradation in the presence of pepsin under appropriate conditions indicates that further analysis should be conducted to determine the likelihood of the newly expressed protein being allergenic. The establishment of a consistent and well validated pepsin degradation protocol may enhance the utility of this method. However, it should be taken into account that a lack of resistance to pepsin does not exclude the possibility that the newly expressed protein could be a relevant allergen. Although the pepsin resistance protocol is recommended, it is recognized that other enzyme susceptibility protocols exist. Alternative protocols may be used where adequate justification is provided[27].

Specific serum screening

For those proteins that originate from a source known to be allergenic, or that have sequence homology with a known allergen, testing in immunological assays should be performed where sera are available. Sera from individuals with a clinically validated allergy to the source of the protein can be used to test the specific binding to IgE class antibodies of the protein in *in vitro* assays. A critical issue for testing will be the availability of human sera from sufficient numbers of individuals[28]. In addition, the quality of the sera and the assay procedure need to be standardized to produce a valid test result. For proteins from sources not known to be allergenic, and which do not exhibit sequence homology to a known allergen, targeted serum screening may be considered where such tests are available, as described in the final paragraph below.

In the case of a newly expressed protein derived from a known allergenic source, a negative result in *in vitro* immunoassays may not be considered sufficient, but should prompt additional testing, such as the possible use of skin test and *ex vivo* protocols[29]. A positive result in such tests would indicate a potential allergen.

[24] FASTA is a computer program, based on the method of W. Pearson and D. Lipman (*Proc. Natl. Acad. Sci. USA*, 85: 2444–2448, 1988), that searches for similarities between one sequence (the query) and any group of sequences (the database) (http://fasta.bioch.virginia.edu/). The BLAST (basic local alignment search tool) program uses a strategy based on matching sequence fragments by employing a powerful statistical model, developed by S. Karlin and S. Altschul (*Proc. Natl. Acad. Sci. USA*, 87: 2264–2268, 1990), to find the best local alignments. BLASTP is the NCBI BLAST program for comparing a protein query sequence to a protein database. The original BLAST program was developed at NCBI (http://www.ncbi.nih.gov/BLAST/). There is a separate BLAST distribution called WU-BLAST available from Washington University (http://blast.wustl.edu/).

[25] It is recognized that the 2001 FAO/WHO consultation suggested moving from eight to six identical amino acid segments in searches. The smaller the peptide sequence used in the stepwise comparison, the greater the likelihood of identifying false positives; conversely, the larger the peptide sequence used, the greater the likelihood of false negatives, thereby reducing the utility of the comparison (FAO/WHO, 2001).

[26] The method outlined in the United States Pharmacopoeia (1995) was used in the establishment of the correlation (Astwood *et al.* 1996).

[27] Report of the Joint FAO/WHO Expert Consultation on allergenicity of foods derived from biotechnology (FAO/WHO, 2001): Section 6.4 Pepsin resistance.

[28] According to the Joint Report of the FAO/WHO Expert Consultation on allergenicity of foods derived from biotechnology (FAO/WHO, 2001) a minimum of eight relevant sera is required to achieve 99 percent certainty that the new protein is not an allergen, in the case of a major allergen. Similarly, a minimum of 24 relevant sera is required to achieve the same level of certainty in the case of a minor allergen. It is recognized that these quantities of sera may not be available for testing purposes.

[29] An *ex vivo* procedure is described as testing for allergenicity performed using cells or tissue culture from allergic human subjects (FAO/WHO, 2001).

The level of exposure to the newly expressed protein and the effects of relevant food processing will contribute towards an overall conclusion about the potential human health risk. In this regard, the nature of the food product intended for consumption should be taken into consideration in determining the types of processing that would be applied and its effects on the presence of the protein in the final food product.

As scientific knowledge and technology evolves, other methods and tools may be considered in assessing the allergenicity potential of newly expressed proteins as part of the assessment strategy. These methods should be scientifically sound and may include targeted serum screening (i.e. the assessment of protein binding to IgE in sera of individuals with clinically validated allergic responses to broadly related categories of foods), the development of international serum banks, use of animal models, and examination of newly expressed proteins for T-cell epitopes and structural motifs associated with allergens.

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Additional resources

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8. Compositional analyses of key components, evaluation of metabolites, food processing and nutritional modification

Compositional analysis

Food composition analysis is concerned with both beneficial and harmful components in the human diet: nutrients, bioactive non-nutrients, antinutrients, toxicants, contaminants and other potentially useful and dangerous elements. The composition of any food varies, and the differences are caused by plant variety, growth and storage conditions, climate, processing and several other factors. As a result, compositional data are used mainly as an estimate or starting point to guide further analysis, if deviations from expectations are seen.

Possible changes in the composition of the recombinant-DNA plant are assessed using comparative analyses of the key nutrients, antinutrients, toxicants and other important components of the crop with the corresponding compounds in an appropriate comparator crop. Data on the composition of recombinant-DNA plants and their conventional counterparts are obtained from samples produced in controlled field trials and analysed using validated methods and appropriate statistical techniques. Samples are normally analysed in a random order using the same methods in order to prevent bias.

Based on the comparative approach, it is important to decide which nutrients the evaluation should be focused on. Generally, the food safety assessment considers the potential for any change in the concentration of key elements that have a significant impact on the diet, as well as the potential for any change in the bioavailability of key nutritional components.

Key compositional data that are statistically non-distinguishable collected from both the recombinant-DNA crop plant and the isogenic counterpart, grown under near identical conditions, are essential to establish substantial equivalence. Moreover, the compositional data should be shown to fall within the published range for conventional varieties that are considered to be safe for consumption based on a history of safe use.

Where significant changes are detected, analytical methods traditionally applied in the evaluation of food constituents, such as measurement of total protein, fat, ash, fibre and micronutrients, may need to be augmented with additional analyses to identify the nature of the changes observed, and to determine whether the observed differences could adversely affect health. Paragraphs 44 to 46 of the Codex Guideline outline the key considerations for key components and metabolites in recombinant-DNA plants.

There may be instances where reference values are not available for a particular food crop e.g. crops that are nutritionally modified and/or indigenous to a specific region. In such cases, the purpose of the assessment is to gather data to establish a compositional profile. It is important to note that all plant breeding methods, conventional and modern, have the potential to alter the compositional profile and nutritional value of plants or lead to unexpected or unintended changes in concentrations of various natural toxicants or antinutrients[30].

Unintended changes in levels of nutrients can theoretically arise in several ways. Insertion of genetic material could disrupt or alter the expression of normally expressed plant genes. Expression of the introduced gene - through protein synthesis - might lead to enzymatic activity and substrate ranges beyond the intended target molecule, and a high transgene expression

[30] International Food Composition Tables Directory, see "additional resources" section.

CODEX GUIDELINE PARAGRAPH 44. Analyses of concentrations of key components[31] of the recombinant-DNA plant, and especially those typical of the food, should be compared with an equivalent analysis of a conventional counterpart grown and harvested under the same conditions. In some cases, a further comparison with the recombinant-DNA plant grown under its expected agronomic conditions may need to be considered (e.g. application of an herbicide). 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. The comparator(s) used in this assessment should ideally be the near isogenic parental line.
In practice, this may not be feasible at all times, in which case a line as close as possible should be chosen. The purpose of this comparison, in conjunction with an exposure assessment as necessary, is to establish that substances that are nutritionally important or that can affect the safety of the food have not been altered in a manner that would have an adverse impact on human health.

CODEX GUIDELINE PARAGRAPH 45. The location of trial sites should be representative of the range of environmental conditions under which the plant varieties would be expected to be grown. The number of trial sites should be sufficient to allow accurate assessment of compositional characteristics over this range. Similarly, trials should be conducted over a sufficient number of generations to allow adequate exposure to the variety of conditions met in nature. To minimise environmental effects, and to reduce any effect from naturally occurring genotypic variation within a crop variety, each trial site should be replicated. An adequate number of plants should be sampled and the methods of analysis should be sufficiently sensitive and specific to detect variations in key components.

CODEX GUIDELINES PARAGRAPH 46. Some recombinant-DNA plants may have been modified in a manner that could result in new or altered levels of various metabolites in the food. Consideration should be given to the potential for the accumulation of metabolites in the food that would adversely affect human health. Safety assessment of such plants requires investigation of residue and metabolite levels in the food and assessment of any alterations in nutrient profile. 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).

level might reduce the availability of amino acids used for synthesis of other compounds. Finally, either the expressed protein or altered levels of other proteins or metabolites might have antinutritional effects[32].

In general, to assess the effects (if any) of a novel protein expressed in a recombinant-DNA plant a number of key parameters are selected: (i) prior history of safe use of the protein in food; (ii) knowledge of the mode of action e.g. enzyme function; (iii) digestibility of the protein in *in vitro* models; (iv) absence of amino acid sequence similarity to sequences in available databases of known mammalian protein toxins and protein allergens or pharmacologically active proteins; (v) predictable levels of expression of the newly introduced protein.

For recombinant-DNA plants that were not developed to have intentionally altered nutritional value, the aim of the nutritional evaluation is to demonstrate that there has been no unintentional change in the levels of key nutrients, natural toxicants or antinutrients, or in the bioavailability of nutrients. In this case, food substitution using products from the recombinant-DNA plants should not adversely affect the health or nutritional status of the consumer. Implications for the population as a whole and for specific subgroups (e.g. children and the elderly) should be considered.

Nevertheless, information on the composition of many plant species is limited, especially with regard to the antinutrient and natural toxin profiles. Because of this, compositional analysis is often hampered when used as a screening method for unintended effects of genetic modification. It is necessary to develop alternative analytical methods that are more informative in these cases. More advanced methodologies, such as mRNA fingerprinting and metabolomic analysis, are being developed but remain to be validated as alternative means of detecting important differences in gene expression and establishing the toxicological significance of the alteration.

Metabolites are dependent on the nutrient profile of a food, which is assessed using the following steps: compositional analysis, morphological and physiological analysis in the form of *in vitro* tests, animal studies and clinical analysis through human studies. Because a broad

31 Key nutrients or key antinutrients are those components in a particular food that may have a substantial impact in the overall diet. They may be major constituents (fats, proteins, carbohydrates as nutrients or enzyme inhibitors as antinutrients) or minor compounds (minerals, vitamins). Key toxicants are those toxicologically significant compounds known to be inherently present in the plant, such as those compounds whose toxic potency and level may be significant to health (e.g. solanine in potatoes if the level is increased, selenium in wheat) and allergens.

32 Changes in gene expression will also occur when conventional breeding methods are used. Unintended changes in plant composition have been argued to be less frequent in recombinant-DNA plants because only a limited number of genes are transferred during the genetic modification process.

selection is made of nutritionally relevant compounds, and known antinutritional and toxic compounds, the targeted analytical approach, i.e. measuring the content of single substances, offers the assurance that unintentional alterations in plant metabolic pathways will be detected. Where changes in plant metabolites raise significant safety concerns, it may be possible to test their safety individually, or when they are present as a component of the food derived from the recombinant-DNA plant.

The basic information required for recombinant-DNA plants includes measurement of various carbohydrates, proteins, fats, energy and water (Greenfield and Southgate, 1996). Data on key vitamins and minerals are required where deficiencies are a cause of disease and for nutritionally modified foods.

The measurement of carbohydrates (McCleary *et al.*, 2006) can be conducted by various means: (i) analytical methods, which measure total starch, resistant starch and dietary fibre; (ii) chemical – the enzymatic degradation of polysaccharides or oligosaccharides to basic sugars; (iii) physical methods, which assess the food structure retained or conferred; (iv) an assessment of functional properties, such as whether the product is glycaemic, digestible, fermentable, etc.

Amino acid analyses are used to determine the protein content of novel foods. This can be achieved by using the Kjeldahl method (or similar) (Association of Official Analytical Chemistry, 2002), which in principle measures the nitrogen content in order to determine the protein content[33]. Alternatively, relying on their structure, proteins can be hydrolysed to their component amino acids, which can then be measured by ion-exchange, gas-liquid or high-performance liquid chromatography. The sum of the amino acids then represents the protein content (by weight) of the food.

Most of the fat in food is in the form of triglycerides. Fats are analysed either as fatty acids and the result expressed as triglycerides or are measured as the fraction of the food that is soluble in lipid solvents.

Food processing

Processing methods can cause a significant variation in the nutrient content of a food compared with the nutrient profile of the crop as it was grown in a field (Morris *et al.*, 2004).

Modern separation techniques, such as milling, centrifugation, and pressing, change the nutritional content of food, preserving certain nutrients while removing others. Because of reduced nutritional value, processed foods are often "enriched" or "fortified" with some of the most critical nutrients (usually certain vitamins) that were lost during processing. Nonetheless, processed foods tend to have an inferior nutritional profile to whole, fresh foods, with respect to the content of sugar, starch, potassium/sodium, vitamins, fibre, and intact, unoxidized (essential) fatty acids. In addition, processed foods often contain potentially harmful substances such as oxidized fats and trans-fatty acids.

Heating techniques may reduce the content of many heat-labile nutrients such as certain vitamins and phytochemicals, and possibly other as yet undiscovered substances. For example, boiling a potato can cause a significant amount of the potato's B and C vitamins to be lost through an osmotic reaction between the potato and the boiling water. Similar losses occur when food is roasted or fried in oil. The actual nutrient losses observed are affected by many factors such as food type, cooking time and temperature.

CODEX GUIDELINE PARAGRAPH 47. The potential effects of food processing, including home preparation, on foods derived from recombinant-DNA plants 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 ingredient from the plant. For example, in the case of vegetable oil, information should be provided on the extraction process and any subsequent refining steps.

33 This approach is based on two assumptions: that dietary carbohydrates and fats do not contain nitrogen and that nearly all of the nitrogen in the diet is present as amino acids in proteins.

Nutritional modification

For recombinant-DNA plants that were intentionally developed to have altered nutrients, the aim of the nutritional evaluation is to demonstrate that there are no additional unintentional changes in the levels of nutrients, including changes in the bioavailability of these nutrients.

The approach to the safety assessment of products with intentionally modified nutrient profiles is fundamentally the same as for the first generation of recombinant-DNA plants (OECD, 2001). However, the compositional differences between these products and their conventional counterparts are likely to be greater, thus increasing the potential for unintended effects. In essence, the utility of current methods for assessing the safety of recombinant-DNA plants may be found to be limited, due to the fact that the nutritionally modified crops will not be substantially equivalent to their conventional counterparts and will share fewer compositional values for comparison.

Nutritionally modified products may be produced to address a specific dietary or nutritional need. The safety assessment, however, must consider not only the target group but also groups in the population that may be at risk, thus recognizing the presence of population diversity. This requires validated data on food consumption patterns, nutrient intake and in some instances the nutritional status of a population or target group. The safety assessment of a nutritionally modified food must be considered in the context of a total diet.

Due to the potential for broad changes in nutrient levels and interactions with other nutrients, and unexpected effects, it may be necessary in certain instances to undertake feeding

CODEX GUIDELINE PARAGRAPH 48. The assessment of possible compositional changes to key nutrients, which should be conducted for all recombinant-DNA plants, has already been addressed under 'Compositional analyses of key components'. However, foods derived from recombinant-DNA plants that have undergone modification to intentionally alter nutritional quality or functionality should be subjected to additional nutritional assessment 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.

CODEX GUIDELINE PARAGRAPH 49. 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 derived from the recombinant-DNA plant. 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.

CODEX GUIDELINE PARAGRAPH 50. The use of plant breeding, including in *vitro* nucleic acid techniques, to change nutrient levels in crops can result in broad changes to the nutrient profile in two ways. The intended modification in plant constituents could change the overall nutrient profile of the plant product and this change could affect the nutritional status of individuals consuming the food. Unexpected alterations in nutrients could have the same effect. Although the recombinant-DNA plant components may be individually assessed as safe, the impact of the change on the overall nutrient profile should be determined.

CODEX GUIDELINE PARAGRAPH 51. When the modification results in a food product, such as vegetable oil, 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 or food components whose nutritional composition is closer to that of the food derived from recombinant-DNA plant) as appropriate comparators to assess the nutritional impact of the food.

CODEX GUIDELINE PARAGRAPH 52. Because of geographical and cultural variation in food consumption patterns, nutritional changes to a specific food may have a greater impact in some geographical areas or in some cultural population than in others. Some food plants serve as the major source of a particular nutrient in some populations. The nutrient and the populations affected should be identified.

CODEX GUIDELINE PARAGRAPH 53. Some foods may require additional testing. For example, animal feeding studies may be warranted for foods derived from recombinant-DNA plants 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 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.

studies in other animals to determine the outcomes that result from changes in nutrient profiles and nutrient bioavailability.

New analytical methods

Improved methodologies and more sensitive techniques allow detection of unintended alterations in the composition of foods in a way that was once not possible. The application of profiling methods such as DNA/RNA microarray technology, proteomics, gas chromatography coupled to mass spectrometry (GC-MS) and liquid chromatography coupled to nuclear magnetic resonance (HPLC-NMR) has the potential to provide indications of changes at the level of mRNA expression, protein production and/or at the level of metabolism without prior knowledge of specific changes in plant constituents.

The utility and applicability of these non-targeted techniques for risk assessment needs further exploration, in particular with respect to establishing and validating the relevance to food safety of the observed changes. One of the major difficulties is to distinguish between natural variations and variations that have resulted from genetic modification. It is essential that databases of plant constituent profiles under different conditions contain the full range of values of each measured parameter under a wide range of environmental, genetic, and development conditions. This information would need to be correlated with the presence or absence of associated food safety hazards.

Profiling methods are not yet suitable for routine risk assessment purposes, and further development and validation are needed. A more promising application of these methods may currently lie in a hypothesis-driven analysis of relevant categories of compounds that may be altered. Thus profiling methods are not aimed at replacing conventional single compound analyses, but may be useful, when validated, to confirm and supplement other data.

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Additional resources

International Life Sciences Institute (ILSI). Crop Composition Database. A comprehensive online crop composition database that provides up-to-date information on the natural variability in the composition of conventional crops and provides a reference for comparing the composition of new crop varieties, including those developed through biotechnology. http://www.cropcomposition.org/

See also: **ILSI.** 2003. *Best practices for the conduct of animal studies to evaluate crops genetically modified for input traits*. Washington, DC, ILSI Press. http://www.ilsi.org/AboutILSI/IFBIC/BESTPRACTICES.htm

FAO INFOODS. The International Food Data Systems Project (INFOODS) is a comprehensive effort, begun within the UN University's Food and Nutrition Programme, to improve data on the nutrient composition of foods from all parts of the world, with the goal of ensuring that adequate and reliable data can be obtained and interpreted properly worldwide. http://www.fao.org/infoods/directory_en.stm

OECD. 1998. *Report of the OECD workshop on the toxicological and nutritional testing of novel foods*. Paris, Organization for Economic Co-operation and Development (OECD).

USDA National Nutrient Database for Standard Reference. The Nutrient Data Laboratory (NDL) has the responsibility to develop the USDA's National Nutrient Database for Standard Reference, the foundation of most food and nutrition databases in the United States, which is used in determining food policy, research and nutrition monitoring. http://www.nal.usda.gov/fnic/foodcomp/search ●

9. Perspectives on safety assessment of foods derived from the next generation of recombinant-DNA plants

Introduction

In recent years, the genetic alterations in new plant varieties under development have become more complex, with more genes involved and with an increasing tendency to alter existing metabolic pathways or even introduce new ones. These "second generation" recombinant-DNA plants have been deliberately modified to express novel traits to enhance nutrition and health (e.g. increased vitamin levels) or to improve livestock feed. Unlike the first generation of recombinant-DNA crops, which were not intended to have altered nutritional properties and whose single-gene traits were relatively straightforward to assess for safety, these second generation products may be intentionally designed not to be substantially equivalent to their conventional counterparts. This may introduce new challenges for those tasked with assessing the safety of foods and feedstuffs derived from these recombinant-DNA plants as there may be no conventional comparator against which the foods derived from the recombinant-DNA plants can be measured.

The next generation of recombinant-DNA plants is likely to be genetically more complex (and may blur the boundary between foods and therapeutics e.g. nutraceuticals, edible vaccines and biopharmaceuticals). This will make the application of the concept of substantial equivalence less appropriate, and the safety assessment of such products is likely to depend on additional approaches to assessment and parallel improvements in the understanding of the relationship between plant composition and health impacts. Ensuring that the safety assessment takes into account the dietary needs and consumption patterns of potentially affected (sub)populations will be vital.

It is anticipated that GM food products that have been modified to be significantly different from their conventional counterparts will receive different, if not greater, scrutiny than those GM foods that have been approved by regulatory authorities to date. New analytical methods for predicting and assessing these differences are being considered (reviewed by Kuiper and Kleter, 2003). The utility of these methods is constrained at present by the fact that insufficient data are available to indicate if statistically significant differences that may be identified using profiling methods such as DNA or RNA microarrays or proteomics are biologically relevant from a safety standpoint.

Internationally, few attempts have been made to examine how best to assess the safety of GM foods with enhanced nutritional or health benefits. The International Life Sciences Institute has published a document that provides the scientific underpinnings and recommendations for assessing the safety and nutritional effects of crops with improved nutritional qualities (the document does not address GM foods that offer potential health benefits). It includes terms and definitions for describing such products, identifies the key safety and nutritional challenges, and introduces potential approaches and methods to address those challenges (ILSI, 2004). A more recent initiative has been undertaken by the Codex *ad hoc* Intergovernmental Task Force on Foods Derived from Biotechnology. In 2005, the Task Force agreed to initiate new work to develop an annex to its 2003 Guideline (see Appendix 2). The annex will elaborate on the guidance provided in the 2003 Guideline.

General principles for the addition of essential nutrients to foods

The Codex Alimentarius Commission (CAC, 2007) provides the guidance for the maintenance or improvement of the overall nutritional quality of foods through the addition of essential nutrients for the purposes of fortification, restoration and nutritional equivalence. The General Principles also address the addition of essential nutrients to special purpose foods to ensure an adequate and appropriate nutrient content. The General Principles aim to prevent the indiscriminate addition of essential nutrients to foods, thereby decreasing the risk of health hazard due to nutrient excesses, deficits or imbalances. The Codex introduced these general principles in 1987 with subsequent amendments. Internationally, there is increased understanding of nutrient enhanced or health promoting foods. It is however a scientifically more involved field of research requiring a different treatment compared with providing a crop variety with increased resistance to a disease, insect pest or herbicide, even when biotechnological tools are utilized for the purpose.

The general principles review by CAC (2007) focused on:

1. new methods of achieving addition or enhancement of the levels of essential nutrients in foods, including biofortification;
2. the need for additional approaches to controlling the addition of essential nutrients to foods, including discretionary fortification;
3. the addition to foods of bioactive substances.

Biofortification

The Codex review (2007) defines biofortification as the indirect addition of essential nutrients or 'other substances' to foods for the purpose of nutritional enhancement or health enhancement. In addition to direct addition of the nutrient to foods at the time of processing, it is possible indirectly to add the nutrients at an earlier point of food production. Genetic transformation using recombinant-DNA technology is one such tool, using which the plant or animal is enabled to produce the additional nutrient e.g. an enhanced beta-carotene level in rice (see Box 9.1).

Box 9.1. Golden rice

An example of this new generation of recombinant-DNA plants is the development of "Golden Rice" in an international network involving the European Union, India, the Philippines, Vietnam and Bangladesh (http://www.goldenrice.org). Dietary micronutrient deficiencies, such as the lack of vitamin A, are a major source of morbidity (increased susceptibility to disease) and mortality worldwide. This deficiency affects particularly children, impairing their immune systems and normal development, causing disease and ultimately death. The best way to avoid micronutrient deficiencies is by way of a varied diet, rich in vegetables, fruits and animal products. According to the WHO, dietary vitamin A deficiency (VAD) causes some 250 000 to 500 000 children to go blind each year. For people who live below the poverty line in many parts of the world, the common foods consumed, such as rice, need to provide vitamin A. However, rice plants produce β-carotene (provitamin A) in green tissues only and not in the endosperm (the edible part of the seed). In Golden Rice, genes have been inserted into the rice genome by genetic engineering that account for the production and accumulation of β-carotene in the grains. The intensity of the golden colour is an indicator of the concentration of β-carotene in the endosperm. After the concept was proved in 1999, new rice lines with higher _-β-carotene content have been generated and are undergoing field trials. The risk analysis and regulation processes are being followed by adhering to the national system and the Codex guidelines in each country. The goal is to be capable of providing the recommended daily allowance of vitamin A - in the form of β-carotene – in 100–200 g of rice, which corresponds to the daily rice consumption of children in rice-based societies, such as in India, Vietnam or Bangladesh. In other countries, *Golden Rice* could still be a valuable complement to children's diets, thus contributing to the reduction of clinical and subclinical VAD-related diseases. *Golden Rice* is a product that does not create new dependencies or displace traditional cuisine and has the potential to save a large number of people from VAD-related diseases.

Box 9.2. Key features of biosafety considerations for nutritionally enhanced foods

a) Estimation of potential exposure distribution patterns: how to go about determining potential exposure distribution patterns in both target and non-target populations of a country and evaluate the safety of such exposure in vulnerable groups. Although techniques are available to simulate such patterns using modeling, it is felt that there is no substitute for controlled trials and investigations, first in animals, and then in consenting, informed humans. In this perspective, an important social issue needs to be taken care of, which is to label the foods derived from recombinant-DNA in the marketplace to provide a means of identifying the GM foods in epidemiological studies as part of post-release monitoring and risk management.

b) Bioavailability: bioavailability analysis should be incorporated into regulatory reviews of all recombinant-DNA plants developed for enhanced nutrition or health. Bioavailability studies using cell cultures have been recommended before feeding trials are taken up and employ radiolabelled compounds to trace the fate of the nutrient upon metabolism in a cell (Wood and Tamura, 2001).

c) Upper limits of safe intake: the need to determine upper limits of safe intake for the nutrient or bioactive substance is essential to eliminate any risk associated with excessive intake of the food. Upper limits of foods containing enhanced nutrients have to be determined for each targeted nutrient as different nutrients can have different upper limits for their safe intake in human beings. Recombinant-DNA plants with modifications of nutrients need to be clearly identified and efforts taken to prevent their use without informed consent. Safe upper limits of ingestion need to be established using the pure form of the targeted nutrient followed by the edible form of the foodstuffs, first in animals then in human volunteers.

d) Stability testing of novel proteins introduced into the recombinant-DNA derived crop as a foodstuff needs to be undertaken because the novel products may create unexpected toxic by-products, especially when the primary plant product is processed into different forms and preparations. The behaviour of these proteins, if unknown from other sources of food, must be subjected to testing in processing as well as storage, in addition to the toxicity testing of the product.

Based on the models developed in Canada (Health Canada, 2005), by the European Commission (EC, 2006), and by Rasmussen *et al.* (2006), an attempt is being made to identify threshold limits for enhanced nutrients so that the risk of indiscriminate addition of essential nutrients is reduced and their effects on health can be evaluated. Similarly, there is a need for further research to identify nutrient-wise (case by case) the minimum levels of addition of nutrients to a biofortified food so that its desired consequence is realized beyond the discernible effect, to enable properly informed labelling of the product.

The Independent Science Panel, launched in 2003 in the United Kingdom[34], has discussed the issue of biosafety of nutritionally enhanced transgenic foods in response to the questionnaire developed by the Codex targeting recombinant-DNA derived foods aimed at nutritional or health benefits. Some key features of the biosafety considerations for nutritionally enhanced foods and crops are described in Box 9.2.

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10. Risk communication among stakeholders

Introduction

Risk communication is one of the three distinct but closely linked components of risk analysis as defined by the Codex Alimentarius Commission[35]. It is "the interactive exchange of information and opinions throughout the risk analysis process concerning risk, risk-related factors and risk perceptions, among risk assessors, risk managers, consumers, industry, the academic community and other interested parties, including the explanation of risk assessment findings and the basis of risk management decisions". Along with risk assessment and risk management, risk communication is integral to the overall risk analysis of a food derived from recombinant-DNA plants. Risk communication is the science of understanding scientific and technological risk and how it is communicated within a sociopolitical structure (Powell, 2000).

The processes of assessing the risks involved, and the methods of managing them whilst focusing on health and the safety of the environment, need to be communicated in a simple comprehensive manner without getting into the depths of the technological details involved. It is useful to make it clear to the stakeholder that if a GM crop has a bacterial gene for a specific protein, it does not mean that the transformed crop will be harbouring the bacterium itself, but it only means that the crop now has the capability of producing the new protein from within its own physiology using the gene that was originally present in the bacterium. Once this is established, the communication details should focus on the various regulatory processes involved in ensuring the safe deployment of the technology and its benefits to the end users.

35 *Working principles for risk analysis for application in the framework of the Codex Alimentarius* (adopted by the 26th Session of the Codex Alimentarius Commission, 2003; Codex Alimentarius Commission Procedural Manual; 13th edition)

Box 10.1. Risk communication in the process of risk analysis

1. promote awareness and understanding of the specific issues under consideration during the risk analysis;
2. promote consistency and transparency in formulating risk management options/recommendations;
3. provide a sound basis for understanding the risk management decisions proposed;
4. improve the overall effectiveness and efficiency of the risk analysis;
5. strengthen the working relationships among participants;
6. foster public understanding of the process, so as to enhance trust and confidence in the safety of the food supply;
7. promote the appropriate involvement of all interested parties;
8. exchange information in relation to the concerns of interested parties about the risks associated with food.

Essential features of risk communication

The Codex Alimentarius Commission (2003) lists the characteristics that should be included in risk communication in the process of risk analysis (Box 10.1).

The major function of risk communication should be to ensure that all information and opinions required for effective risk management are incorporated into the decision-making process. It should include a transparent explanation of the risk assessment policy and of the assessment of risk, including the uncertainty. The need for specific standards or related texts and the procedures followed to determine them, including how the uncertainty was dealt with, should also be clearly explained. It should indicate any constraints, uncertainties, assumptions and their impact on the risk analysis, and minority opinions that have been expressed in the course of the risk assessment. However, even though it is expected to be transparent and accessible to all interested parties, if there are legitimate concerns to

preserve confidentiality, these should be respected while information on the risk analysis should be made available.

Risk communication is an important part of the biosafety procedures that ensure public acceptance of food derived from recombinant-DNA plants. To communicate and interact with the public at large about the specific risks and the actions taken to alleviate them before the recombinant-DNA crop reaches the field or the derived food reaches the markets is a crucial step in reassuring the stakeholders. It is also a mechanism that builds confidence among the stakeholders in a gradual manner, moving along with the different phases of the development of the recombinant-DNA plant and the foods derived from it. In the absence of this channel, a void gets created leading to the stakeholders not being made aware of the efforts taken by the regulators to reduce the risks assessed with the technology. This also encourages the spread of fictitious stories from not fully informed individuals to others, along with their own potentially misleading messages.

Media coverage of food derived from recombinant-DNA plants can become polarized into safety versus risk; science moving forward versus science out of control; competitiveness versus safety (Powell and Leiss, 1997). Media analysis is a tool used to help understand the formation of public opinion and to look at what people are saying and what they are being told. This reliance on the media helps to define the public's sense of reality (Nelkin, 1987) and their perceptions of risks or benefits.

Risk communication can be divided into two major components: technical components that generally comprise the scientific hazards evaluated in the risk assessment and the management options arising out of the assessment, and non-technical components that include the administrative protocols, and the cultural and ethical issues in society as dealt with by the regulatory agencies during the process of risk analysis.

Regulatory risk communication

Regulatory risk communication starts primarily by keeping the stakeholder groups (the whole food chain involving scientist, farmer, trader, processor, product developer, market player [retailer] and consumer) informed of the upcoming technology as soon as the technology development project for a particular crop is approved by an institution. From this stage onwards, methods need to be devised for comprehensible transmission of information at various stages of product development until it reaches the markets, so that the primary stakeholder at each stage is taken into confidence.

It is important that only accurate information should be given, as risk communication tends to influence psychological and cultural beliefs. Assessment of the scientific risks must be coupled with appropriate research-based risk management and communication activities to provide consumers, the media and others with a balanced, science-based assessment of both

CODEX PRINCIPLES PARAGRAPH 22. Effective risk communication is essential at all phases of risk assessment and risk management. It is an interactive process involving all interested parties, including government, industry, academia, media and consumers.

CODEX PRINCIPLES PARAGRAPH 23. Risk communication should include transparent safety assessment and risk management decision-making processes. These processes should be fully documented at all stages and open to public scrutiny, whilst respecting legitimate concerns to safeguard the confidentiality of commercial and industrial information. In particular, reports prepared on the safety assessments and other aspects of the decision-making process should be made available to all interested parties.

CODEX PRINCIPLES PARAGRAPH 24. Effective risk communication should include responsive consultation processes. Consultation processes should be interactive. The views of all interested parties should be sought and relevant food safety and nutritional issues that are raised during consultation should be addressed during the risk analysis process.

the potential benefits and the risks of a particular technology, and to positively impact the development of public policy. The challenge is to incorporate public perceptions into policy development without abdicating the leadership role of science.

Risk communication is addressed in the Codex Principles for the Risk Analysis of Foods Derived from Modern Biotechnology (see Appendix 1) as follows.

Risk communication is used to explain both how and why decisions are taken. It specifically acknowledges any concerns raised by stakeholders, including the public, and explains how these concerns have been addressed. This captures the reality that risk communication is an iterative exchange between interested and affected parties that primarily, but not exclusively, focuses on risks. In practice, because of the wide diversity of stakeholders involved in agricultural biotechnology, risk communication is largely a non-technical dialogue about both real and perceived risks.

It is widely recognized that more could – and should – be done to make information concerning the safety assessment of novel foods available to the public. This has become more important with increased consumer interest in the safety of food derived from recombinant-DNA plants. The OECD countries and intergovernmental organizations are looking for new ways to share their experiences. They are promoting information dissemination and sound understanding of the safety issues on the part of consumers (OECD, 2000). A number of countries have adopted measures concerned with sharing information on the safety assessment of GM foods with the public. These include:

a. inviting public comments on reports containing safety evaluations by scientific assessment bodies;
b. disclosure of data used in safety assessments to support applications;
c. publication of results of meetings of safety assessment bodies.

Regulatory authorities are actively involving, and consulting, the public with regard to food safety and regulation. Some authorities have a policy of full disclosure of the information contained in applications (except for confidential commercial information). The Internet is increasingly used to make information on safety assessment and approval procedures available to the public. It is a good source of information on crops and other foods that have been approved. Some countries are exploring the potential of the Internet to make details of applications more widely available, in order to make the assessment process as open, transparent and inclusive as possible.

The OECD's BioTrack Online site (http://www.oecd.org/ehs/service.htm) is a valuable source of information on regulatory developments in Member countries. It includes information on responsible ministries or agencies, and details of laws, regulations and guidelines. There are also two important databases, one of products that have been commercialized, and the other of field trials of GM crops that have taken place in OECD countries.

Risk communication as a two-way process

Regulatory risk communication deals with providing information about the regulatory framework and processes designed to assess and manage risk, such as policy development, application processes, stakeholder participation, product-specific decisions, and access to the information that is used to inform regulatory decision-making. In order to avoid real or perceived conflicts of interest many regulatory agencies undertake only regulatory risk communication activities and leave more technology- or product-focused communication efforts to other stakeholder groups. As much effort should be put into gathering input and feedback as into giving out information.

To be effective, regulatory risk communicators need to devise appropriate mechanisms to receive feedback, analyse it and use the information to revise and improve their communication outreach. Obtaining feedback and input from stakeholders enables regulators and risk assessors

Box 10.2. Useful considerations in risk communication

Know the audience
Before formulating risk communication messages, the audience should be analysed to understand their motivations and opinions. Beyond knowing in general who the audience is, it is necessary actually to get to know them as groups, and ideally as individuals, to understand their concerns and feelings and to maintain an open channel of communication with them. Listening to all interested parties is an important part of risk communication.

Involve the scientific experts
Scientific experts, in their capacity as risk assessors, must be able to explain the concepts and processes of risk assessment. They need to be able to explain the results of their assessment and the scientific data, assumptions and subjective judgements upon which it is based, so that risk managers and other interested parties clearly understand the risk. They must also be able to communicate clearly what they know and what they do not know, and to explain the uncertainties related to the risk assessment process. In turn, the risk managers must be able to explain how the risk management decisions have been arrived at.

Establish expertise in communication
Successful risk communication requires expertise in conveying understandable and usable information to all interested parties. Risk managers and technical experts may not have the time or the skill to perform complex risk communication tasks, such as responding to the needs of the various audiences (public, industry, media, etc.) and preparing effective messages. People with expertise in risk communication should therefore be involved as early as possible in the process. This expertise will probably have to be developed by training and experience.

Be a credible source of information
Information from credible sources is more likely to influence the public perception of a risk than is information from sources that lack this attribute. The credibility accorded to a source by a target audience may vary according to the nature of the hazard, culture, social and economic status, and other factors. If consistent messages are received from multiple sources then the credibility of the message is reinforced. Factors determining source credibility include recognized competence or expertise, trustworthiness, fairness and lack of bias. For example, the terms that consumers have associated with high credibility include factual, knowledgeable, expert, public welfare, responsible, truthful and good "track record". Trust and credibility must be nurtured, and it can be eroded or lost through ineffective or inappropriate communication. In studies, consumers have indicated that distrust and low credibility result from exaggeration, distortion and perceived vested interest.

Effective communications acknowledge current issues and problems, are open in their content and approach, and are timely. Timeliness of the message is most important because many controversies become focused on the question "why didn't you tell us sooner?", rather than on the risk itself. Omissions, distortions and self-serving statements will damage credibility in the longer term.

Share responsibility
Regulatory agencies of governments at the national, regional and local levels have a fundamental responsibility for risk communication. The public expects the government to play a leading role in managing public health risks. This is true when the risk management decision involves regulatory or voluntary controls, and is even true when the government decision is to take no action. In the latter event, communication is still essential to provide the reasons why taking no action is the best option. In order to understand public concerns and to ensure that risk management decisions respond to those concerns in appropriate ways, the government needs to determine what the public knows about the risks and what the public thinks of the various options being considered to manage those risks.

The media play an essential role in the communication process and therefore share these responsibilities. Communication on immediate risks involving human health, particularly when there is a potential for serious health consequences, such as with food-borne illnesses, cannot be treated in the same manner as less immediate food safety concerns. Industry also has a responsibility for risk communication, especially when the risk is as a result of their products or processes. All parties involved in the risk communication process (e.g. government, industry, media) have joint responsibilities for the outcome of that communication, even though their individual roles may differ. Because science must be the basis for decision-making, all parties involved in the communication process should know the basic principles and data supporting the risk assessment and the policies underlying the resulting risk management decisions.

Differentiate between science and value judgement
It is essential to separate "facts" from "values" in considering risk management options. At a practical level, it is useful to report the facts that are known at the time as well as the uncertainties that are involved in the risk management decisions being proposed or implemented. The risk communicator bears the responsibility to explain what is known as fact and where the limits of this knowledge begin and end. Value judgements are involved in the concept of acceptable levels of risk. Consequently, risk communicators should be able to justify the level of acceptable risk to the public. Many people take the term "safe food" to mean food with zero risk, but zero risk is often unattainable. In practice, "safe food" usually means food that is "safe enough". Making this clear is an important function of risk communication.

Assure transparency
For the public to accept the risk analysis process and its outcomes, the process must be transparent. While respecting legitimate concerns to preserve confidentiality (e.g. proprietary information or data), transparency in risk analysis consists of having the process open and available for scrutiny by interested parties. Effective two-way communication between risk managers and the public and other interested parties is both an essential part of risk management and a key to achieving transparency.

to identify and address stakeholder concerns. Often the best route for information dissemination involves strengthening existing information channels. For example, if governments publish progress updates in local newspapers, mechanisms to use this for agricultural biotechnology risk communication may be best in the short term. However, if governments rely only on mechanisms such as "Government Gazettes", which are poorly distributed, to inform the public then attention needs to be paid to alternative ways of disseminating information to and receiving it from the target groups.

Credibility is often built into a communication process by providing technical reviews of the process in simple language. For example, reviews can be commissioned that explain the science and technology involved in the process and the regulatory procedures involved (Beever and Kemp, 2000).

Different audiences or stakeholder groups have different needs and so it is important to understand an audience well before designing communication for them. Identifying an audience's needs, concerns, knowledge level, opinions and preferred mechanisms for communicating through consultation supports the development of a communication style that will be effective.

The type of audience should also be carefully considered when selecting the best communicators. Effective communicators need to be credible and trusted, and different people may be required for different target groups. In addition communicators need to have excellent language and listening skills. In general, the credibility of communicators depends on cultural norms and differs from society to society and between sectors.

Two targeted questions that need to be answered during risk communication are: "are foods from recombinant-DNA plants safe?" and "what foods have been genetically modified?". This raises the issue of choice and knowing what foods from recombinant-DNA plants may be in the marketplace. In order to address these questions, regulatory authorities typically make information available about the national regulatory framework that identifies the competent authorities; details the regulatory requirements for the different stages in product development (e.g. research and development, confined or experimental field testing, and premarket safety assessments); explains how safety assessments are conducted, and clearly indicates how decisions are made, including opportunities for public participation in decision-making and the factors taken into account by decision-makers. The feedback is also put within a time frame so that any additional information or clarification can be provided to interested parties.

Additionally, many regulatory authorities publish product-specific decision summaries that provide information about specific transgenic events.

The report of a joint FAO/WHO Expert Consultation on the application of risk communication to food standards and safety matters provides a helpful summary of principles for risk communication that are applicable to those involved in communicating about the regulation and safety assessment of foods from recombinant-DNA plants[36].

Risk communication in safety assessment

Although most countries attempt to provide complete and clear information on the foods derived from recombinant-DNA plants, the information itself is often found to be too complex and multidisciplinary in nature to be understood fully by the public without bias or ambiguity. The challenge is to present the material in a suitable form for different audiences without compromising the accuracy of the information. It is necessary to make the message as communicative as possible to enable the consumer to make an informed choice on accepting the food derived from recombinant-DNA plants with reference to the risks associated with it. The Canadian Biotechnology Advisory Committee (CBAC, 2002) considered the options listed below.

a. **Creation of better information about the regulatory system.** An initial step may be to improve the description and communication of information about the Canadian food regulatory

[36] FAO. 1999. *The application of risk communication to food standards and safety matters.* FAO Food and Nutrition Paper 70. Rome, Food and Agriculture Organization. http://www.fao.org/docrep/005/x1271e/X1271E00.HTM

system for GM and other novel foods, and to ensure that the material provided is complete, understandable and easily retrievable. A variety of media (for example, the Internet, booklets, articles) could be used to make the information more widely available. The material could be presented with various levels of complexity to be helpful to different readers.

b. **Creation of a centralized information body.** A centralized body for consumer information on food biotechnology could provide information on food production, GM foods and other novel food biotechnology, relevant laws and regulations, scientific knowledge, perspectives on ethical and social issues, ongoing research and activities, and how to contribute to government-related activities. In addition to discussing the traditional foods and plant-breeding practices, an attempt should be made to provide a meaningful description of the benefits, risks and uncertainties associated with different types of foods.

c. **Increase public awareness and engagement.** In addition to the above options, a proactive communications programme may be useful for increasing public awareness. Opportunities for Canadians to comment on various aspects of GM foods could be provided through public dialogue sessions.

The Biotechnology Consortium of India Limited (BCIL) is another such communication portal and is a unique combination of public–private partnership providing all the technical information and social concerns with respect to biosafety assessment on recombinant-DNA research and commercial activities. Developed on the pattern of the biosafety clearing house concept, it also undertakes to conduct workshops in different parts of the country in an open forum involving all stakeholders and regulatory agencies on specific issues (BCIL, 2007). For interested parties, hyperlinks or downloadable access to self-contained reviews may be provided to enable an informed understanding among stakeholders on the safety issues, and effective management strategies.

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11. Glossary of terms, links and resources

The following terms frequently appear in dossiers submitted for safety evaluation. For more information on biotechnology-related terminology, see the FAO Glossary of Biotechnology for Food and Agriculture at http://www.fao.org/biotech/index_glossary.asp

Glossary

Adjuvant
An agent mixed with an antigen that enhances the immune response to that antigen or to immunization.

Antisense gene
A gene that produces a transcript (mRNA) that is complementary to the pre-mRNA or mRNA of a normal gene (usually constructed by inverting the coding region relative to the promoter).

Bioavailability
The proportion of a nutrient or administered drug, etc. that can be taken up by an organism in a biologically effective form. For example, some soils high in phosphorus (P) have a low level of P availability because the pH of the soil renders much of the P insoluble.

Biosafety
Refers to the avoidance of risk to human health and safety, and to the conservation of the environment, during the use for research and commerce of infectious or genetically modified organisms.

Biotechnology (modern)
The application of:
1. *In vitro* nucleic acid techniques, including recombinant deoxyribonucleic acid (DNA) and direct injection of nucleic acid into cells or organelles; or
2. Fusion of cells beyond the taxonomic family that overcome natural physiological reproductive or recombination barriers and that are not techniques used in traditional breeding and selection (Cartagena Protocol on Biosafety to the Convention on Biological Diversity).

Biotechnology (traditional)
1. Any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use (Convention on Biological Diversity).
2. Interpreted in a narrow sense, which considers only the new DNA techniques, molecular biology and reproductive technological applications, biotechnology covers a range of different technologies such as gene manipulation and gene transfer, DNA typing and cloning of plants and animals (FAO statement on biotechnology).

Comparative approach
The comparative approach, previously referred to as substantial equivalence, embodies the concept that GM foods can be assessed to a large extent by comparison to the benchmark of commonly consumed foods already regarded as safe (the traditional or non-modified counterpart). The comparison is usually made at the level of the composition of the food.

Concatemer
A DNA segment made up of repeated sequences linked head to tail.

Concatenation
Combination of two (or more) strings of DNA in a defined order.

Conventional counterpart
A related plant variety, its components and/or products for which there is experience of establishing safety based on common use as food.

Copy number
The number of copies of a particular plasmid per bacterial cell, or copies of a gene per genome.

Dietary exposure
Contact by ingestion between a physical, chemical or biological agent and an organism.

Gene silencing
Gene silencing is a general term describing epigenetic processes of gene regulation and refers to an event of interruption or suppression of the expression of a gene. Genes are regulated at either the transcriptional or post-transcriptional level. Transcriptional gene silencing is the result of histone modifications, creating an environment of heterochromatin around a gene that makes it inaccessible to transcriptional machinery. Post-transcriptional gene silencing is the result of mRNA of a particular gene being destroyed. The destruction of the mRNA prevents translation to form an active gene product. The term frequently appears in the dossiers often refers to a natural reaction of plants to high levels of foreign gene expression. However, not all foreign gene expression leads to gene silencing. Many factors contribute to gene silencing including the nature and orientation of the foreign transgenes, expression levels and phase of development.

Genetic engineering
Modification of the genotype, and hence the phenotype, by transgenesis, which is the introduction of a gene or genes into animal or plant cells, which leads to the transmission of the input gene (transgene) to successive generations.

Genetically modified foods (GM foods)
Genetically modified (GM) foods are foods produced from genetically modified organisms (GMOs) that have had their genome altered through genetic engineering (e.g. GM corn) or foods that contain ingredients from GMOs (e.g. chocolate containing GM soybeans).

Genetically modified organism (GMO)
An organism that has been transformed by the insertion of one or more transgenes.

Hapten
A small molecule, which by itself is not an antigen, but which as a part of a larger structure when linked to a carrier protein, can serve as an antigenic determinant.

Helper plasmid
A plasmid that provides a function or functions to another plasmid in the same cell.

Immunoglobulin E (IgE)
Class E immunoglobulins (IgE) are highly specialized antibodies that are produced in lymphatic tissue near the respiratory and digestive tracts. Although they make up only 0.001 percent of antibodies, IgE immunoglobulins are involved in virtually every allergic reaction. IgE antibodies dock onto their respective allergen and stimulate the production of substances that cause inflammation. The subsequent immune over-reaction is known as an allergy. Specialized IgE antibodies can be detected in the blood serum of individuals who are sensitive to the respective allergen.

In vitro digestibility assay
Methods are available for determining the digestibility of protein-containing composites, including foods and feed ingredients. The methods comprise incubation of the composite with proteases, followed by determination of the hydrolysed peptide bonds. The methods are suitable for rapid, routine determination of digestibility in food and feed processing plants.

Isogenic parental line
In genetically modified plants, isogenic initial lines mean those non-GM plants from which the GM strains are derived. Thus, the only difference between GM plants and their derivative isogenic line will be those genes that have been transferred transgenically. Evaluating GM plants for possible unexpected effects necessitates comparison with unmodified parental strains. In order to eliminate any possible influence of normal genetic variation between different hereditary lines and varieties, isogenic lines are usually used as a standard for comparison.

Open reading frame (ORF)
A sequence of nucleotides in a DNA molecule that has the potential to encode a peptide or protein. An ORF contains a start triplet (ATG), which is followed by a series of triplets (each of which encodes an amino acid), and ends with a stop codon (TAA, TAG or TGA). The term is generally applied to sequences of DNA fragments for which no function has yet been determined. The number of ORFs provides an estimate of the number of genes transcribed from the DNA sequence.

Outcrossing
A mating between different populations or individuals of the same species that are not closely related. The term "outcrossing" can be used to describe unintended pollination by an outside source of the same crop during hybrid seed production.

Pleiotropy (pleiotropic effects)
The simultaneous effect of a given gene on more than one apparently unrelated trait.

Positional effect
The influence of the location of a gene (particularly a transgene) on its expression and hence on the phenotype.

Post-translational modification
The addition of specific chemical residues to a protein after it has been translated. Common residues are phosphate groups (phosphorylation) and sugars (glycosylation).

Recombinant
A term used in both classical and molecular genetics.
1. In classical genetics: an organism or cell that is the result of meiotic recombination.
2. In molecular genetics: a hybrid molecule made up of DNA obtained from different organisms. Typically used as an adjective, e.g. recombinant-DNA.

Recombinant-DNA
The result of combining DNA fragments from different sources.

Substantial equivalence
Substantial equivalence is a concept, first described in an OECD publication in 1993, which stresses that an assessment of a novel food, in particular one that is genetically modified, should demonstrate that the food is as safe as its traditional counterpart.

Toxicokinetics
The study of the time-dependent processes related to toxicants as they interact with living organisms. It encompasses absorption, distribution, storage, biotransformation and elimination.

Transfer DNA (T-DNA)
The DNA segment of the Ti plasmid, present in pathogenic Agrobacterium tumefaciens, that is transferred to plant cells and inserted into the plant's DNA as part of the infection process. Wild-type T-DNA encodes enzymes that induce the plant to synthesize specific opines that are required for bacterial growth. In engineered T-DNAs, these genes are replaced by one or more transgenes.

Transgene
An isolated gene sequence used to transform an organism. Often, but not always, the transgene has been derived from a different species from that of the recipient.

Weediness
The ability of a plant to colonize a disturbed habitat and compete with cultivated species.

Links and resources

Inter-governmental organizations

Food and Agriculture Organization
The multi-lingual FAO Biotechnology website provides access to updated news and events, documents, an e-mail forum, a glossary, national biotechnology policy documents and other useful information about many aspects of modern biotechnology.
http://www.fao.org/biotech

Codex Alimentarius
The Codex Alimentarius Commission was created in 1963 by FAO and WHO to develop food standards, guidelines and related texts such as codes of practice under the Joint FAO/WHO Food Standards Programme. Related to GM food safety, the Codex ad hoc Intergovernmental Task Force on Foods Derived from Biotechnology has published *Principles for the risk analysis of foods derived from modern biotechnology* and *Guideline for the conduct of food safety assessment of foods derived from recombinant-DNA plants*, provided in Appendices 1 and 2 of this monograph. http://www.codexalimentarius.net/web/index_en.jsp

World Health Organization

WHO has been addressing a wide range of issues in the field of biotechnology and human health, including safety evaluation of vaccines produced using biotechnology, human cloning and gene therapy. http://www.who.int/foodsafety/biotech/en/

Organisation for Economic Co-operation and Development

The OECD's programme of work for the Safety of Novel Foods and Feeds is intended to promote international harmonization in the safety assessment and regulation of GM foods and feeds, including the products of modern biotechnology. The OECD's Task Force for the Safety of Novel Foods and Feeds decided at its first session, in 1999, to focus its work on the development of science-based consensus documents, which are mutually acceptable among member countries. These consensus documents contain information for use during the regulatory assessment of a particular food/feed product. In the area of food and feed safety, consensus documents are being published on the nutrients, antinutrients or toxicants, information on the product's use as a food/feed and other relevant information.
http://www.oecd.org/topic/0,2686,en_2649_37437_1_1_1_1_37437,00.html

Biosafety Clearing House

The Biosafety Clearing-House (BCH) is an information exchange mechanism established by the Cartagena Protocol on Biosafety to assist Parties to implement its provisions and to facilitate sharing of information on, and experience with, living modified organisms (LMOs).
http://bch.biodiv.org/

International Centre for Genetic Engineering and Biotechnology

ICGEB offers a rich array of information. The BioSafety web page provides extensive links to international treaties, conventions and meetings, including submissions by member governments. http://www.icgeb.org

United Nations Industrial Development Organization

UNIDO is the only organization that maintains detailed databases of key industrial statistics with worldwide coverage. It has established a network of regional centres providing comprehensive training in biosafety. http://binas.unido.org/wiki/index.php/Main_Page

Institute for Health and Consumer Protection of the Joint Research Center

IHCP is part of the Directorate General JRC and fulfils the JRC's mission in providing scientific support to policies related to health and consumer protection. http://ihcp.jrc.ec.europa.eu/

Some Governmental regulatory web sites related to GM foods

Australia and New Zealand

Food Safety Australia New Zealand (FSANZ).
http://www.foodstandards.gov.au/foodmatters/gmfoods/index.cfm

Canada

Health Canada.
http://www.hc-sc.gc.ca/food-aliment/mh-dm/ofb-bba/nfi-ani/e_novel_foods_and_ingredient.html

European Commission

European Food Safety Authority (EFSA).
http://www.efsa.europa.eu/en/science/gmo.html

India

Department of Biotechnology: Biosafety Rules and Regulations.
http://dbtbiosafety.nic.in/

Japan

Ministry of Health, Labour and Welfare.
http://www.mhlw.go.jp/english/topics/food/index.html

United States

Food and Drug Administration, http://www.cfsan.fda.gov/~lrd/biotechm.html#reg
United States Department of Agriculture, http://www.usda.gov
United States Environmental Protection Agency, Office of Prevention, Pesticides and Toxic Substances, http://www.epa.gov/ ●

Appendices

Relevant Codex documents

Appendix 1.
Principles for the Risk Analysis of Foods Derived from Modern Biotechnology CAC/GL 44-2003

Section 1 – Introduction

1. For many foods, the level of food safety generally accepted by the society reflects the history of their safe consumption by humans. It is recognised that in many cases the knowledge required to manage the risks associated with foods has been acquired in the course of their long history of use. Foods are generally considered safe, provided that care is taken during development, primary production, processing, storage, handling and preparation.

2. The hazards associated with foods are subjected to the risk analysis process of the Codex Alimentarius Commission to assess potential risks and, if necessary, to develop approaches to manage these risks. The conduct of risk analysis is guided by general decisions of the Codex Alimentarius Commission[1] as well as the Codex Working Principles for Risk Analysis[2].

3. While risk analysis has been used over a long period of time to address chemical hazards (*e.g.* residues of pesticides, contaminants, food additives and processing aids), and it is being increasingly used to address microbiological hazards and nutritional factors, the principles were not elaborated specifically for whole foods.

4. The risk analysis approach can, in general terms, be applied to foods including foods derived from modern biotechnology. However, it is recognised that this approach must be modified when applied to a whole food rather than to a discrete hazard that may be present in food.

5. The principles presented in this document should be read in conjunction with the Codex Working Principles for Risk Analysis to which these principles are supplemental.

6. Where appropriate, the results of a risk assessment undertaken by other regulatory authorities may be used to assist in the risk analysis and avoid duplication of work.

Section 2 – Scope and definitions

7. The purpose of these Principles is to provide a framework for undertaking risk analysis on the safety and nutritional aspects of foods derived from modern biotechnology. This document does not address environmental, ethical, moral and socio-economic aspects of the research, development, production and marketing of these foods[3].

8. The definitions below apply to these Principles:

- "Modern Biotechnology" means the application of:
  - i) *In vitro* nucleic acid techniques, including recombinant deoxyribonucleic acid (DNA) and direct injection of nucleic acid into cells or organelles, or
  - ii) Fusion of cells beyond the taxonomic family, that overcome natural physiological reproductive or recombinant barriers and that are not techniques used in traditional breeding and selection[4].
- "Conventional Counterpart" means a related organism/variety, its components and/or products for which there is experience of establishing safety based on common use as food[5].

Section 3 – Principles

9. The risk analysis process for foods derived from modern biotechnology should be consistent with the Codex Working Principles for Risk Analysis.

Risk assessment

10. Risk assessment includes a safety assessment, which is designed to identify whether a hazard,

1 These decisions include the Statements of principle concerning the role of science in the Codex decision-making process and the extent to which other factors are taken into account and the Statements of principle relating to the role of food safety risk assessment (Codex Alimentarius Commission Procedural Manual; Thirteenth edition).

2 "Working Principles for Risk Analysis for Application in the Framework of the Codex Alimentarius"(adopted by the 26th Session of the Codex Alimentarius Commission, 2003; Codex Alimentarius Commission Procedural Manual; Thirteenth edition)

3 This document does not address animal feed and animals fed such feed except insofar as these animals have been developed by using modern biotechnology.

4 This definition is taken from the Cartagena Biosafety Protocol under the Convention on Biological Diversity.

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

nutritional or other safety concern is present, and if present, to gather information on its nature and severity. The safety assessment should include a comparison between the food derived from modern biotechnology and its conventional counterpart focusing on determination of similarities and differences. If a new or altered hazard, nutritional or other safety concern is identified by the safety assessment, the risk associated with it should be characterized to determine its relevance to human health.

11. A safety assessment is characterized by an assessment of a whole food or a component thereof relative to the appropriate conventional counterpart:

A) taking into account both intended and unintended effects;
B) identifying new or altered hazards;
C) identifying changes, relevant to human health, in key nutrients.

12. A pre-market safety assessment should be undertaken following a structured and integrated approach and be performed on a case-by-case basis. The data and information, based on sound science, obtained using appropriate methods and analysed using appropriate statistical techniques, should be of a quality and, as appropriate, of quantity that would withstand scientific peer review.

13. Risk assessment should apply to all relevant aspects of foods derived from modern biotechnology. The risk assessment approach for these foods is based on a consideration of science-based multidisciplinary data and information taking into account the factors mentioned in the accompanying Guidelines[6].

14. Scientific data for risk assessment are generally obtained from a variety of sources, such as the developer of the product, scientific literature, general technical information, independent scientists, regulatory agencies, international bodies and other interested parties. Data should be assessed using appropriate science-based risk assessment methods.

15. Risk assessment should take into account all available scientific data and information derived from different testing procedures, provided that the procedures are scientifically sound and the parameters being measured are comparable.

Risk management

16. Risk management measures for foods derived from modern biotechnology should be proportional to the risk, based on the outcome of the risk assessment and, where relevant, taking into account other legitimate factors in accordance with the general decisions of the Codex Alimentarius Commission[7] as well as the Codex Working Principles for Risk Analysis.

17. It should be recognised that different risk management measures may be capable of achieving the same level of protection with regard to the management of risks associated with safety and nutritional impacts on human health, and therefore would be equivalent.

18. Risk managers should take into account the uncertainties identified in the risk assessment and implement appropriate measures to manage these uncertainties.

19. Risk management measures may include, as appropriate, food labelling[8] conditions for marketing approvals and post-market monitoring.

20. Post-market monitoring may be an appropriate risk management measure in specific circumstances. Its need and utility should be considered, on a case-by-case basis, during risk assessment and its practicability should be considered during risk management. Post-market monitoring may be undertaken for the purpose of:

A) verifying conclusions about the absence or the possible occurrence, impact and significance of potential consumer health effects; and
B) monitoring changes in nutrient intake levels, associated with the introduction of foods likely to significantly alter nutritional status, to determine their human health impact.

21. Specific tools may be needed to facilitate the implementation and enforcement of risk management measures. These may include appropriate analytical methods; reference materials; and, the tracing of products[9] for the purpose of facilitating withdrawal from the market when a risk to human health has been

6 Reference is made to the Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants (CAC/GL 45-2003) and the Guideline for the Conduct of Food Safety Assessment of Foods Produced using Recombinant-DNA Microorganisms (CAC/GL 46-2003).

7 See footnote 1.

8 Reference is made to the CCFL in relation to the Proposed Draft Guidelines for the Labelling of Foods and Food Ingredients obtained through certain techniques of genetic modification/genetic engineering at Step 3 of the Codex Elaboration Procedure.

9 It is recognised that there are other applications of product tracing. These applications should be consistent with the provisions of the SPS and TBT Agreements. The application of product tracing to the areas covered by both Agreements is under consideration within Codex on the basis of decisions of 49th Session of Executive Committee.

identified or to support post-market monitoring in circumstances as indicated in paragraph 20.

Risk communication

22. Effective risk communication is essential at all phases of risk assessment and risk management. It is an interactive process involving all interested parties, including government, industry, academia, media and consumers.

23. Risk communication should include transparent safety assessment and risk management decision-making processes. These processes should be fully documented at all stages and open to public scrutiny, whilst respecting legitimate concerns to safeguard the confidentiality of commercial and industrial information. In particular, reports prepared on the safety assessments and other aspects of the decision-making process should be made available to all interested parties.

24. Effective risk communication should include responsive consultation processes. Consultation processes should be interactive. The views of all interested parties should be sought and relevant food safety and nutritional issues that are raised during consultation should be addressed during the risk analysis process.

Consistency

25. A consistent approach should be adopted to characterise and manage safety and nutritional risks associated with foods derived from modern biotechnology. Unjustified differences in the level of risks presented to consumers between these foods and similar conventional foods should be avoided.

26. A transparent and well-defined regulatory framework should be provided in characterising and managing the risks associated with foods derived from modern biotechnology. This should include consistency of data requirements, assessment frameworks, the acceptable level of risk, communication and consultation mechanisms and timely decision processes.

Capacity building and information exchange

27. Efforts should be made to improve the capability of regulatory authorities, particularly those of developing countries, to assess, manage and communicate risks, including enforcement, associated with foods derived from modern biotechnology or to interpret assessments undertaken by other authorities or recognised expert bodies, including access to analytical technology. In addition capacity building for developing countries either through bilateral arrangements or with assistance of international organizations should be directed toward effective application of these principles[10].

28. Regulatory authorities, international organisations and expert bodies and industry should facilitate through appropriate contact points including but not limited to Codex Contact Points and other appropriate means, the exchange of information including the information on analytical methods.

Rewiew processes

29. Risk analysis methodology and its application should be consistent with new scientific knowledge and other information relevant to risk analysis.

30. Recognizing the rapid pace of development in the field of biotechnology, the approach to safety assessments of foods derived from modern biotechnology should be reviewed when necessary to ensure that emerging scientific information is incorporated into the risk analysis. When new scientific information relevant to a risk assessment becomes available the assessment should be reviewed to incorporate that information and, if necessary, risk management measures adapted accordingly ●

10 Reference is made to technical assistance of provisions in Article 9 of the SPS Agreement and Article 11 of the TBT Agreement.

Appendix 2.
Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants CAC/GL 45-2003

Section 1 – Scope

1. This Guideline supports the Principles for the Risk Analysis of Foods Derived from Modern Biotechnology. It addresses safety and nutritional aspects of foods consisting of, or derived from, plants that have a history of safe use as sources of food, and that have been modified by modern biotechnology to exhibit new or altered expression of traits.

2. This document does not address animal feed or animals fed with the feed. This document also does not address environmental risks.

3. 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 a specific chemical or microbial contaminant that have identifiable hazards and risks; they are not intended to apply to whole foods as such. Indeed, few foods have been assessed scientifically in a manner that would fully characterise all risks associated with the food. Further, many foods contain substances that would likely be found 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. This approach is based on the principle that the safety of foods derived from new plant varieties, including recombinant-DNA plants, is assessed relative to the conventional counterpart having a history of safe use, taking into account both intended and unintended effects. Rather than trying to identify every hazard associated with a particular food, the intention is to identify new or altered hazards relative to the conventional counterpart.

5. 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 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.

6. 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 Principles for the Risk Analysis of Foods derived from Modern Biotechnology.

7. The Guideline describes the recommended approach to making safety assessments of foods derived from recombinant-DNA plants where a conventional counterpart exists, and identifies the data and information that are generally applicable to making such assessments. While this Guideline is designed for foods derived from recombinant- DNA plants, the approach described could, in general, be applied to foods derived from plants that have been altered by other techniques.

Section 2 – Definition

8. The definitions below apply to this Guideline:

- "Recombinant-DNA Plant"– means a plant 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" – means a related plant variety, its components and/or products for which there is experience of establishing safety based on common use as food[1].

Section 3 – Introduction to food safety assessment

9. Traditionally, new varieties of food plants have not been systematically subjected to extensive chemical, toxicological, or nutritional evaluation prior to marketing, with the exception of foods for specific groups, such as infants, where the food may constitute a substantial portion of the diet. Thus, new varieties of corn, soya, potatoes and other common food plants are evaluated by breeders for agronomic and phenotypic characteristics, but generally, foods derived from such

[1] It is recognized that for the foreseeable future, foods derived from modern biotechnology will not be used as conventional counterparts.

new plant varieties are not subjected to the rigorous and extensive food safety testing procedures, including studies in animals, that are typical of chemicals such as food additives or pesticide residues that may be present in food.

10. The use of animal models for assessing toxicological endpoints 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 characterised, 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.

11. Animal studies cannot readily be applied to testing the risks associated with whole foods, which are complex mixtures of compounds, often characterised 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 which 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 foods. 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.

12. 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 derived from food plants, including recombinant- DNA plants. This has been addressed by the development of a multidisciplinary approach for assessing safety which takes into account both intended and unintended changes that may occur in the plant or in the foods derived from it, using the concept of substantial equivalence.

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. This concept is used to identify similarities and differences between the new food and its conventional counterpart[2]. 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.

Unintended effects

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.

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 enzymes at high levels may give rise to secondary biochemical

[2] 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).

effects or changes in the regulation of metabolic pathways and/or altered levels of metabolites.

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.

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.

Framework of food safety assessment

18. The safety assessment of a food derived from a recombinant-DNA plant follows a stepwise process of addressing relevant factors that include:

A) Description of the recombinant-DNA plant;
B) Description of the host plant and its use as food;
C) Description of the donor organism(s);
D) Description of the genetic modification(s);
E) Characterization of the genetic modification(s);
F) Safety assessment:
  a) expressed substances (non-nucleic acid substances);
  b) compositional analyses of key components;
  c) evaluation of metabolites ;
  d) food processing;
  e) nutritional modification; and
G) Other considerations.

19. In certain cases, the characteristics of the product may necessitate development of additional data and information to address issues that are unique to the product under review.

20. 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 at request. Data should be obtained using sound scientific methods and analysed using appropriate statistical techniques. The sensitivity of all analytical methods should be documented.

21. The goal of each safety assessment is to provide assurance, in the light of the best available scientific knowledge, that the food does not cause harm when prepared, used and/or eaten according to its intended use. 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. In essence, therefore, 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 consideration

Description of the recombinant-DNA plant

22. A description of the recombinant-DNA plant being presented for safety assessment should be provided. This description should identify the crop, the transformation event(s) to be reviewed and the type and purpose of the modification. This description should be sufficient to aid in understanding the nature of the food being submitted for safety assessment.

Description of the host plant and its use as food

23. A comprehensive description of the host plant should be provided. The necessary data and information

should include, but need not be restricted to:

A) common or usual name; scientific name; and, taxonomic classification;

B) history of cultivation and development through breeding, in particular identifying traits that may adversely impact on human health ;

C) information on the host plant's genotype and phenotype relevant to its safety, including any known toxicity or allergenicity; and

D) history of safe use for consumption as food.

24. Relevant phenotypic information should be provided not only for the host plant, but also for related species and for plants that have made or may make a significant contribution to the genetic background of the host plant.

25. The history of use may include information on how the plant is typically cultivated, transported and stored, whether special processing is required to make the plant safe to eat, and the plant's normal role in the diet (*e.g.* which part of the plant is used as a food source, whether its consumption is important in particular subgroups of the population, what important macro- or micro-nutrients it contributes to the diet).

Description of the donor organism(s)

26. Information should be provided on the donor organism(s) and, when appropriate, on other related species. It is particularly important to determine if the donor organism(s) or other closely related members of the family naturally exhibit characteristics of pathogenicity or toxin production, or have other traits that affect human health (*e.g.* presence of anti-nutrients). The description of the donor organism(s) should include:

A) its usual or common name;

B) scientific name;

C) taxonomic classification;

D) information about the natural history as concerns food safety;

E) information on naturally occurring toxins, anti-nutrients and allergens; for microorganisms, additional information on pathogenicity and the relationship to known pathogens; and

F) information on the past and present use, if any, in the food supply and exposure route(s) other than intendedfood use (*e.g.* possible presence as contaminants).

Description of the genetic modification(s)

27. Sufficient information should be provided on the genetic modification to allow for the identification of all genetic material potentially delivered to the host plant and to provide the necessary information for the analysis of the data supporting the characterization of the DNA inserted in the plant.

28. The description of the transformation process should include:

A) information on the specific method used for the transformation (*e.g.*Agrobacterium-mediated transformation);

B) information, if applicable, on the DNA used to modify the plant (*e.g.* helper plasmids), including the source (*e.g.* plant, microbial, viral, synthetic), identity and expected function in the plant; and

C) intermediate host organisms including the organisms (*e.g.* bacteria) used to produce or process DNA for transformation of the host organism.

29. Information should be provided on the DNA to be introduced, including:

A) the characterization of all the genetic components including marker 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)

30. In order to provide clear understanding of the impact on the composition and safety of foods derived from recombinant-DNA plants, a comprehensive molecular and biochemical characterization of the genetic modification should be carried out.

31. Information should be provided on the DNA insertions into the plant genome; this should include:

A) the characterization and description of the inserted genetic materials;

B) the number of insertion sites;

C) the organisation of the inserted genetic material at each insertion site including copy number and sequence data of the inserted material and of the surrounding region, sufficient to identify any substances expressed as a consequence of the inserted material, or, where more appropriate, other information such as analysis of transcripts or

expression products to identify any new substances that may be present in the food; and

D) identification of any open reading frames within the inserted DNA or created by the insertions with contiguous plant genomic DNA including those that could result in fusion proteins.

32. Information should be provided on any expressed substances in the recombinant-DNA plant; this should include:

A) the gene product(s) (*e.g.* a protein or an untranslated RNA);

B) the gene product(s)' function;

C) the phenotypic description of the new trait(s);

D) the level and site of expression in the plant of the expressed gene product(s), and the levels of its metabolites in the plant, particularly in the edible portions; and

E) where possible, the amount of the target gene product(s) if the function of the expressed sequence(s)/gene(s) is to alter the accumulation of a specific endogenous mRNA or protein.

33. In addition, information should be provided:

A) to demonstrate whether the arrangement of the genetic material used for insertion has been conserved or whether significant rearrangements have occurred upon integration;

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 through several generations consistent with laws of inheritance. It may be necessary to examine the inheritance of the DNA insert itself or the expression of the corresponding RNA if the phenotypic characteristics cannot be measured directly;

D) to demonstrate whether the newly expressed trait(s) are expressed as expected in the appropriate tissues in a manner and at levels that are 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 host plant has been affected by the transformation process; and

F) to confirm the identity and expression pattern of any new fusion proteins.

Safety assessment

Expressed Substances (non-nucleic acid substances)

Assessment of possible toxicity

34. *In vitro* nucleic acid techniques enable the introduction of DNA that can result in the synthesis of new substances in plants. The new substances can be conventional components of plant foods such as proteins, fats, carbohydrates or vitamins which are novel in the context of that recombinant-DNA plant. New substances might also include new metabolites resulting from the activity of enzymes generated by the expression of the introduced DNA.

35. The safety assessment should take into account the chemical nature and function of the newly expressed substance and identify the concentration of the substance in the edible parts of the recombinant-DNA plant, including variations and mean values. Current dietary exposure and possible effects on population sub-groups should also be considered.

36. 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 plants that do not normally express those toxic or anti-nutritious characteristics. This assurance is particularly important in cases where a recombinant-DNA plant is processed differently from a donor plant, since conventional food processing techniques associated with the donor organisms may deactivate, degrade or eliminate anti-nutrients or toxicants.

37. For the reasons described in Section 3, 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.

38. 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, lectins) as well as stability to heat or processing and to degradation in appropriate representative gastric and intestinal model systems. Appropriate oral toxicity studies[3] may need to be carried out in cases where the protein present in the food is not similar to proteins that have previously been

[3] Guidelines for oral toxicity studies have been developed in international fora, for example, the OECD Guidelines for the Testing of Chemicals.

consumed safely in food, and taking into account its biological function in the plant where known.

39. Potential toxicity of non-protein substances that have not been safely consumed in food should be assessed on a case-by-case basis depending on the identity and biological function in the plant of the substance and dietary exposure. The type of studies to be performed may include studies on metabolism, toxicokinetics, sub-chronic toxicity, chronic toxicity/carcinogenicity, reproduction and development toxicity according to the traditional toxicological approach.

40. This may require the isolation of the new substance from the recombinant-DNA plant, or the synthesis or production of the substance from an alternative source, in which case, the material should be shown to be biochemically, structurally, and functionally equivalent to that produced in the recombinant-DNA plant.

Assessment of possible allergenicity (proteins)

41. When the protein(s) resulting from the inserted gene is present in the food, it should be assessed for potential allergenicity in all cases. An integrated, stepwise, case-by-case approach used in the assessment of the potential allergenicity of the newly-expressed protein(s) should rely upon various criteria used in combination (since no single criterion is sufficiently predictive on either allergenicity or non-allergenicity). As noted in paragraph 20, the data should be obtained using sound scientific methods. A detailed presentation of issues to be considered can be found in the Annex to this document[4].

42. The newly expressed proteins in foods derived from recombinant-DNA plants should be evaluated for any possible role in the elicitation of gluten-sensitive enteropathy, if the introduced genetic material is obtained from wheat, rye, barley, oats, or related cereal grains.

43. The transfer of genes from commonly allergenic foods and from foods 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.

[4] The FAO/WHO expert consultation 2001 report, which includes reference to several decision trees, was used in developing the Annex to these guidelines.

Compositional Analyses of Key Components

44. Analyses of concentrations of key components[5] of the recombinant-DNA plant and, especially those typical of the food, should be compared with an equivalent analysis of a conventional counterpart grown and harvested under the same conditions. In some cases, a further comparison with the recombinant-DNA plant grown under its expected agronomic conditions may need to be considered (*e.g.* application of an herbicide). 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. The comparator(s) used in this assessment should ideally be the near isogenic parental line.

In practice, this may not be feasible at all times, in which case a line as close as possible should be chosen. The purpose of this comparison, in conjunction with an exposure assessment as necessary, is to establish that substances that are nutritionally important or that can affect the safety of the food have not been altered in a manner that would have an adverse impact on human health.

45. The location of trial sites should be representative of the range of environmental conditions under which the plant varieties would be expected to be grown. The number of trial sites should be sufficient to allow accurate assessment of compositional characteristics over this range. Similarly, trials should be conducted over a sufficient number of generations to allow adequate exposure to the variety of conditions met in nature. To minimise environmental effects, and to reduce any effect from naturally occurring genotypic variation within a crop variety, each trial site should be replicated. An adequate number of plants should be sampled and the methods of analysis should be sufficiently sensitive and specific to detect variations in key components.

Evaluation of Metabolites

46. Some recombinant-DNA plants may have been modified in a manner that could result in new or altered levels of various metabolites in the food. Consideration should be given to the potential for the accumulation of metabolites in the food that would adversely affect

[5] 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 constituents (fats, proteins, carbohydrates as nutrients or enzyme inhibitors as anti-nutrients) or minor compounds (minerals, vitamins). Key toxicants are those toxicologically significant compounds known to be inherently present in the plant, such as those compounds whose toxic potency and level may be significant to health (e.g. solanine in potatoes if the level is increased, selenium in wheat) and allergens.

human health. Safety assessment of such plants requires investigation of residue and metabolite levels in the food and assessment of any alterations in nutrient profile. 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).

Food Processing

47. The potential effects of food processing, including home preparation, on foods derived from recombinant-DNA plants 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 ingredient from the plant. For example, in the case of vegetable oil, information should be provided on the extraction process and any subsequent refining steps.

Nutritional Modification

48. The assessment of possible compositional changes to key nutrients, which should be conducted for all recombinant- DNA plants, has already been addressed under 'Compositional analyses of key components'. However, foods derived from recombinant-DNA plants that have undergone modification to intentionally alter nutritional quality or functionality should be subjected to additional nutritional assessment 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.

49. 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 derived from the recombinant-DNA plant. 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.

50. The use of plant breeding, including *in vitro* nucleic acid techniques, to change nutrient levels in crops can result in broad changes to the nutrient profile in two ways. The intended modification in plant constituents could change the overall nutrient profile of the plant product and this change could affect the nutritional status of individuals consuming the food. Unexpected alterations in nutrients could have the same effect. Although the recombinant-DNA plant components may be individually assessed as safe, the impact of the change on the overall nutrient profile should be determined.

51. When the modification results in a food product, such as vegetable oil, 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 or food components whose nutritional composition is closer to that of the food derived from recombinant-DNA plant) as appropriate comparators to assess the nutritional impact of the food.

52. Because of geographical and cultural variation in food consumption patterns, nutritional changes to a specific food may have a greater impact in some geographical areas or in some cultural population than in others. Some food plants serve as the major source of a particular nutrient in some populations. The nutrient and the populations affected should be identified.

53. Some foods may require additional testing. For example, animal feeding studies may be warranted for foods derived from recombinant-DNA plants 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 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.

Section 5 – Other considerations

Potential accumulation of substances significant to human health

54. Some recombinant-DNA plants may exhibit traits (*e.g.* herbicide tolerance) which may indirectly

result in the potential for accumulation of pesticide residues, altered metabolites of such residues, toxic metabolites, contaminants, or other substances which may be relevant to human health. The safety assessment should take this potential for accumulation into account. Conventional procedures for establishing the safety of such compounds (*e.g.* procedures for assessing the human safety of chemicals) should be applied.

Use of antibiotic resistance marker genes

55. Alternative transformation technologies that do not result in antibiotic resistance marker genes in foods should be used in the future development of recombinant-DNA plants, where such technologies are available and demonstrated to be safe.

56. Gene transfer from plants and their food products to gut microorganisms or human cells is considered a rare possibility because of the many complex and unlikely events that would need to occur consecutively. Nevertheless, the possibility of such events cannot be completely discounted[6].

57. In assessing safety of foods containing antibiotic resistance marker genes, the following factors should be considered:

A) the clinical and veterinary use and importance of the antibiotic in question;
   (Certain antibiotics are the only drug available to treat some clinical conditions (*e.g.* vancomycin for use in treating certain staphylococcal infections). Marker genes encoding resistance to such antibiotics should not be used in recombinant-DNA plants.)
B) whether the presence in food of the enzyme or protein encoded by the antibiotic resistance marker gene would compromise the therapeutic efficacy of the orally administered antibiotic; and
   (This assessment should provide an estimate of the amount of orally ingested antibiotic that could be degraded by the presence of the enzyme in food, taking into account factors such as dosage of the antibiotic, amount of enzyme likely to remain in food following exposure to digestive conditions, including neutral or alkaline stomach conditions and the need for enzyme cofactors (*e.g.* ATP) for enzymatic activity and estimated concentration of such factors in food.)
C) safety of the gene product, as would be the case for any other expressed gene product.

6 In cases where there are high levels of naturally occurring bacteria which are resistant to the antibiotic, the likelihood of such bacteria transferring this resistance to other bacteria will be orders of magnitude higher than the likelihood of transfer between ingested foods and bacteria.

58. If evaluation of the data and information suggests that the presence of the antibiotic resistance marker gene or gene product presents risks to human health, the marker gene or gene product should not be present in the food. Antibiotic resistance genes used in food production that encode resistance to clinically used antibiotics should not be present in foods.

Review of safety assessments

59. The goal of the safety assessment is a conclusion as to whether the new food 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.

Annex 1. Assessment of possible allergenicity

Section 1 – Introduction

1. All newly expressed proteins[7] in recombinant-DNA plants that could be present in the final food should be assessed for their potential to cause allergic reactions. This should include consideration of whether a newly expressed protein is one to which certain individuals may already be sensitive as well as whether a protein new to the food supply is likely to induce allergic reactions in some individuals.

2. At present, there is no definitive test that can be relied upon to predict allergic response in humans to a newly expressed protein, therefore, it is recommended that an integrated, stepwise, case by case approach, as described below, be used in the assessment of possible allergenicity of newly expressed proteins. This approach takes into account the evidence derived from several types of information and data since no single criterion is sufficiently predictive.

3. The endpoint of the assessment is a conclusion as to the likelihood of the protein being a food allergen.

7 This assessment strategy is not applicable for assessing whether newly expressed proteins are capable of inducing glutensensitive or other enteropathies. The issue of enteropathies is already addressed in Assessment of possible allergenicity(proteins), paragraph 42 of the Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant- DNA Plants. In addition, the strategy is not applicable to the evaluation of foods where gene products are down regulated for hypoallergenic purposes.

Section 2 – Assessment strategy

4. The initial steps in assessing possible allergenicity of any newly expressed proteins are the determination of: the source of the introduced protein; any significant similarity between the amino acid sequence of the protein and that of known allergens; and its structural properties, including but not limited to, its susceptibility to enzymatic degradation, heat stability and/or, acid and enzymatic treatment.

5. As there is no single test that can predict the likely human IgE response to oral exposure, the first step to characterize newly expressed proteins should be the comparison of the amino acid sequence and certain physicochemical characteristics of the newly expressed protein with those of established allergens in a weight of evidence approach. This will require the isolation of any newly expressed proteins from the recombinant-DNA plant, 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 plant. Particular attention should be given to the choice of the expression host, since post-translational modifications allowed by different hosts (*i.e.*: eukaryotic *vs.* prokaryotic systems) may have an impact on the allergenic potential of the protein.

6. It is important to establish whether the source is known to cause allergic reactions. Genes derived from known allergenic sources should be assumed to encode an allergen unless scientific evidence demonstrates otherwise.

Section 3 – Initial assessment

Section 3.1 – Source of the protein

7. As part of the data supporting the safety of foods derived from recombinant-DNA plants, information should describe any reports of allergenicity associated with the donor organism. Allergenic sources of genes would be defined as those organisms for which reasonable evidence of IgE mediated oral, respiratory or contact allergy is available.

Knowledge of the source of the introduced protein allows the identification of tools and relevant data to be considered in the allergenicity assessment. These include: the availability of sera for screening purposes; documented type, severity and frequency of allergic reactions; structural characteristics and amino acid sequence; physicochemical and immunological properties (when available) of known allergenic proteins from that source.

Section 3.2 – Amino acid sequence homology

8. The purpose of a sequence homology comparison is to assess the extent to which a newly expressed protein is similar in structure to a known allergen. This information may suggest whether that protein has an allergenic potential. Sequence homology searches comparing the structure of all newly expressed proteins with all known allergens should be done. Searches should be conducted using various algorithms such as FASTA or BLASTP to predict overall structural similarities. Strategies such as stepwise contiguous identical amino acid segment searches may also be performed for identifying sequences that may represent linear epitopes. The size of the contiguous amino acid search should be based on a scientifically justified rationale in order to minimize the potential for false negative or false positive results[8]. Validated search and evaluation procedures should be used in order to produce biologically meaningful results.

9. IgE cross-reactivity between the newly expressed protein and a known allergen should be considered a possibility when there is more than 35% identity in a segment of 80 or more amino acids (FAO/WHO 2001) or other scientifically justified criteria. All the information resulting from the sequence homology comparison between the newly expressed protein and known allergens should be reported to allow a case-by-case scientifically based evaluation.

10. Sequence homology searches have certain limitations. In particular, comparisons are limited to the sequences of known allergens in publicly available databases and the scientific literature. There are also limitations in the ability of such comparisons to detect non-contiguous epitopes capable of binding themselves specifically with IgE antibodies.

11. A negative sequence homology result indicates that a newly expressed protein is not a known allergen and is unlikely to be cross-reactive to known

8 It is recognized that the 2001 FAO/WHO consultation suggested moving from 8 to 6 identical amino acid segments in searches. The smaller the peptide sequence used in the stepwise comparison, the greater the likelihood of identifying false positives, inversely, the larger the peptide sequence used, the greater the likelihood of false negatives, thereby reducing the utility of the comparison.

allergens. A result indicating absence of significant sequence homology should be considered along with the other data outlined under this strategy in assessing the allergenic potential of newly expressed proteins. Further studies should be conducted as appropriate (see also sections 4 and 5). A positive sequence homology result indicates that the newly expressed protein is likely to be allergenic. If the product is to be considered further, it should be assessed using serum from individuals sensitized to the identified allergenic source.

Section 3.3 – Pepsin resistance

12. Resistance to pepsin digestion has been observed in several food allergens; thus a correlation exists between resistance to digestion by pepsin and allergenic potential[9]. Therefore, the resistance of a protein to degradation in the presence of pepsin under appropriate conditions indicates that further analysis should be conducted to determine the likelihood of the newly expressed protein being allergenic. The establishment of a consistent and well-validated pepsin degradation protocol may enhance the utility of this method. However, it should be taken into account that a lack of resistance to pepsin does not exclude that the newly expressed protein can be a relevant allergen.

13. Although the pepsin resistance protocol is strongly recommended, it is recognized that other enzyme susceptibility protocols exist. Alternative protocols may be used where adequate justification is provided[10].

Section 4 – Specific serum screening

14. For those proteins that originate from a source known to be allergenic, or have sequence homology with a known allergen, testing in immunological assays should be performed where sera are available. Sera from individuals with a clinically validated allergy to the source of the protein can be used to test the specific binding to IgE class antibodies of the protein in *in vitro* assays. A critical issue for testing will be the availability of human sera from sufficient numbers of individuals[11]. In addition, the quality of the sera and the assay procedure need to be standardized to produce a valid test result. For proteins from sources not known to be allergenic, and which do not exhibit sequence homology to a known allergen, targeted serum screening may be considered where such tests are available as described in paragraph 17.

15. In the case of a newly expressed protein derived from a known allergenic source, a negative result in *in vitro* immunoassays may not be considered sufficient, but should prompt additional testing, such as the possible use of skin test and *ex vivo* protocols[12]. A positive result in such tests would indicate a potential allergen.

Section 5 – Other considerations

16. The absolute exposure to the newly expressed protein and the effects of relevant food processing will contribute toward an overall conclusion about the potential for human health risk. In this regard, the nature of the food product intended for consumption should be taken into consideration in determining the types of processing which would be applied and its effects on the presence of the protein in the final food product.

17. As scientific knowledge and technology evolves, other methods and tools may be considered in assessing the allergenicity potential of newly expressed proteins as part of the assessment strategy. These methods should be scientifically sound and may include targeted serum screening (i.e. the assessment of binding to IgE in sera of individuals with clinically validated allergic responses to broadly-related categories of foods); the development of international serum banks; use of animal models; and examination of newly expressed proteins for T-cell epitopes and structural motifs associated with allergens.

9 The method outlined in the U.S. Pharmacopoeia (1995) was used in the establishment of the correlation (Astwood et al. 1996).

10 Report of Joint FAO/WHO Expert Consultation on Allergenicity of Foods Derived from Biotechnology (2001): Section "6.4 Pepsin Resistance".

11 According to the Joint Report of the FAO/WHO Expert Consultation on Allergenicity of Foods Derived from Biotechnology (22-25 January 2001, Rome, Italy) a minimum of 8 relevant sera is required to achieve a 99% certainty that the new protein is not an allergen in the case of a major allergen. Similarly, a minimum of 24 relevant sera is required to achieve the same level of certainty in the case of a minor allergen. It is recognized that these quantities of sera may not be available for testing purposes.

12 Ex vivo procedure is described as the testing for allergenicity using cells or tissue culture from allergic human subjects (Report of Joint FAO/WHO Expert Consultation on Allergenicity of Foods derived from Biotechnology).

Annex 2. Food safety assessment of foods derived from recombinant-dna plants modified for nutritional or health benefits

Section 1 – Introduction

1. General guidance for the safety assessment of foods derived from recombinant-DNA plants is provided in the Codex Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants (CAC/GL 45-2003) (Codex Plant Guideline). This Annex provides additional considerations that are specific to foods modified for nutritional or health benefits. The document does not extend beyond a safety assessment and therefore, it does not cover assessment of the benefits themselves or any corresponding health claims, or risk-management[13] measures.

2. The following factors determine whether a recombinant-DNA plant is a recombinant-DNA Plant Modified for Nutritional or Health Benefits, and as such within the scope of this Annex:

a) the recombinant-DNA plant exhibits a particular trait in portion(s) of the plant intended for food use, and;
b) The trait is a result of i) introduction of a new nutrient(s) or related substance(s), or ii) alteration of either the quantity or bioavailability of a nutrient(s) or related substance(s), iii) removal or reduction of undesirable substance(s) (e.g. allergens or toxicants), or iv) alteration of the interaction(s) of nutritional or health relevance of these substances.

Section 2 – Definition

3. The definition below applies to this Annex:

Nutrient[14] – means any substance normally consumed as a constituent of food:

a) which provides energy; or
b) which is needed for growth and development and maintenance of healthy life; or
c) a deficit of which will cause characteristic biochemical or physiological changes to occur.

4. This Annex draws, where appropriate, on the definitions of key nutritional concepts to be found or to be developed in relevant Codex texts, especially those elaborated by the Codex Committee on Nutrition and Foods for Special Dietary Uses.

13 Principles for the Risk Analysis of Foods Derived from Modern Biotechnology (CAC/GL 44-2003, paragraph 19).

14 General Principles for the Addition of Essential Nutrients to Foods (CAC/GL 09-1987).

Section 3 – Food safety assessment

5. The Codex General Principles for the Addition of Essential Nutrients to Foods (CAC/GL 09-1987) are generally applicable to the assessment of food derived from a plant which is modified by increasing the amount of a nutrient(s) or related substance(s) available for absorption and metabolism. The Food Safety Framework outlined within the Codex Plant Guideline[15] applies to the overall safety assessment of a food derived from a recombinant-DNA plant modified for nutritional or health benefits. This Annex presents additional considerations regarding the food safety assessment of those foods.

6. Foods derived from recombinant-DNA plants modified for nutritional or health benefits may benefit certain populations/sub populations, while other populations/sub populations may be at risk from the same food[16].

7. Rather than trying to identify every hazard associated with a particular food, the intention of a safety assessment of food derived from recombinant-DNA plants is the identification of new or altered hazards relative to the conventional counterpart[17]. Since recombinant-DNA plants modified for nutritional or health benefits result in food products with a composition that may be significantly different from their conventional counterparts, the choice of an appropriate comparator[18] is of great importance for the safety assessment addressed in this Annex. Those alterations identified in a plant modified to obtain nutritional or health benefits are the subject of this safety assessment.

8. Upper levels of intake for many nutrients that have been set out by some national, regional and international bodies[19] may be considered, as appropriate. The basis for their derivation should also be considered in order to assess the public health implications of exceeding these levels.

9. The safety assessment of related substances should follow a case-by-case approach taking into account upper levels as well as other values, where appropriate.

15 Paragraphs 18-21 (Safety Framework) and 48-53 (Nutrition Modification).

16 Further guidance for susceptible and high-risk population groups is provided in paragraph 49 of the Codex Plant Guideline.

17 Codex Plant Guideline, paragraph 4 .

18 Codex Plant Guideline, paragraph 51.

19 Where such guidance is not provided by Codex, information provided by the FAO/WHO may be preferably considered.

10. Although it is preferable to use a scientifically-determined upper level of intake of a specific nutrient or related substance, when no such value has been determined, consideration may be given to an established history of safe use for nutrients or related substances that are consumed in the diet if the expected or foreseeable exposure would be consistent with those historical safe levels.

11. With conventional fortification of food, typically a nutrient or a related substance is added at controlled concentrations and its chemical form is characterized. Levels of plant nutrients or related substances may vary in both conventionally bred and recombinant-DNA plants due to growing conditions. In addition, more than one chemical form of the nutrient might be expressed in the food as a result of the modification and these may not be characterized from a nutrition perspective. Where appropriate, information may be needed on the different chemical forms of the nutrient(s) or related substance(s) expressed in the portion of the plant intended for food use and their respective levels .

12. Bioavailability of the nutrient(s), related substance(s), or undesirable substance(s) in the food that were the subject of the modification in the recombinant-DNA plant should be established, where appropriate. If more than one chemical form of the nutrient(s) or related substance(s) is present, their combined bioavailability should be established, where appropriate.

13. Bioavailability will vary for different nutrients, and methods of testing for bioavailability should be relevant to the nutrient, and the food containing the nutrient, as well as the health, nutritional status and dietary practices of the specific populations consuming the food. *In vitro* and *in vivo* methods to determine bioavailability exist, the latter conducted in animals and in humans. *In vitro* methods can provide information to assess extent of release of a substance from plant tissues during the digestive process. *In vivo* studies in animals are of limited value in assessing nutritional value or nutrient bioavailability for humans and would require careful design in order to be relevant. In vivo studies, in particular, human studies may provide more relevant information about whether and to what extent the nutrient or related substance is bioavailable.

14. Guidance on dietary exposure assessment of foods derived from recombinant-DNA plants with nutritional modifications is provided in paragraph 49 of the Codex Plant Guideline. In the context of this Annex, dietary exposure assessment is the estimation of the concentration of the nutrient(s) or related substance(s) in a food, the expected or foreseeable consumption of that food, and any known factors that influence bioavailability. Exposure to a nutrient(s) or related substance(s) should be evaluated in the context of the total diet and the assessment should be carried out based on the customary dietary consumption, by the relevant population(s), of the corresponding food that is likely to be displaced. When evaluating the exposure, it is appropriate to consider information on whether the consumption of the modified food could lead to adverse nutritional effects as compared to consumption of the food that it is intended to replace. Most, if not all, aspects of exposure assessment are not unique to recombinant-DNA plants modified for nutritional or health benefits[20].

15. The first step of an exposure assessment is determining the level(s) of the substance(s) in question in the portion of the plant intended for food use. Guidance on determining changes in levels of these substances is provided in the Codex Plant Guideline.[21]

16. Consumption patterns will vary from country to country depending on the importance of the food in the diet(s) of a given population(s). Therefore, it is recommended that consumption estimates are based on national or regional food consumption data when available, using existing guidance on estimation of exposure in a given population(s)[22]. When national or regional food consumption data is unavailable, food availability data may provide a useful resource[23].

17. To assess the safety of a food derived from a recombinant-DNA plant modified for a nutritional or health benefit, the estimated intake of the nutrient or related substance in the population(s) is compared with the nutritional or toxicological reference values, such as upper levels of intake, ADIs for that nutrient or related substance, where these values exist. This may involve assessments of different consumption scenarios against the relevant nutritional reference value, taking into account possible changes in bioavailability, or extend to probabilistic methods that characterise the distribution of exposures within the relevant population(s).

20 Additional applicable guidance on dietary exposure assessment of nutrients and related substances is provided in the Report of a Joint FAO/WHO Technical Workshop on Nutrient Risk Assessment. WHO Headquarters, Geneva, Switzerland, 2-6 May 2005.

21 Paragraphs 44 and 45.

22 A Model for Establishing Upper Levels of Intake for Nutrients and Related Substances. Report of a Joint FAO/WHO Technical Workshop on Nutrient Risk Assessment. WHO Headquarters, Geneva, Switzerland, 2-6 May 2005.

23 Data on staple food products may also be supplemented by information from FAO Food Balance Sheets.

Annex 3. Food safety assessment in situations of low-level presence of recombinant-dna plant material in food

Section 1 – Preamble

1. An increasing number of recombinant–DNA plants are being authorized for commercialization. However, they are authorized at different rates in different countries. As a consequence of these asymmetric authorizations, low levels of recombinant DNA plant materials that have passed a food safety assessment according to the Codex Guideline for the conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants (CAC/GL 45-2003) (Codex Plant Guideline) in one or more countries may on occasion be present in food in importing countries in which the food safety of the relevant recombinant-DNA plants has not been determined.

2. This Annex describes the recommended approach to the food safety assessment in such situations of low-level presence of recombinant-DNA plant material or in advance preparation for such potential circumstances[24].

3. This Annex also describes data and information sharing mechanisms to facilitate utilization of the Annex and to determine whether it should apply.

4. This Annex can be applied in two different dietary exposure situations:

a) That involving commodities, such as grains, beans or oil seeds, in which exposure to food from a variety not authorized in the importing country would likely be to dilute low level amounts at any one time. This would likely be the more common situation of low-level presence of recombinant-DNA plant material. Because any food serving of grains, beans or oil seeds would almost necessarily come from multiple plants, and because of how these types of commodities generally are sourced from multiple farms, are commingled in grain elevators, are further commingled in export shipments, at import and when used in processed foods, any inadvertently commingled material derived from recombinant-DNA plant varieties would be present only at a low level in any individual serving of food.

b) That involving foods that are commonly consumed whole and undiluted, such as some fruits and vegetables like potatoes, tomatoes, and papaya, in which exposure would be rare but could be to an undiluted form of the unauthorized recombinant-DNA plant material. While the likelihood of consuming material from such an unauthorized variety would be low and the likelihood of repeated consumption would be much lower, any such consumption might be of an entire unauthorized fruit or vegetable.

5. In both cases, the dietary exposure will be significantly lower than would be considered in a food safety assessment of the recombinant-DNA plant according to the Codex Plant Guideline. As a result, only certain elements of the Codex Plant Guideline will be relevant and therefore are included in this Annex.

6. This Annex does not:

- address risk management measures; national authorities will determine when a recombinant-DNA plant material is present at a level low enough for this Annex to be appropriate;
- preclude national authorities from conducting a safety assessment according to the Codex Plant Guideline; countries can decide when and how to use the Annex within the context of their regulatory systems; or
- eliminate the responsibility of industries, exporters and, when applicable, national competent authorities to continue to meet countries' relevant import requirements, including in relation to unauthorized recombinant-DNA plant material.

Section 2 – General and other considerations

7. For the food safety assessment in situations of low-level presence of recombinant DNA plant materials in food, sections 4 and 5 of the Codex Plant Guideline apply as amended as follows. The applicable paragraphs are specifically indicated. Those paragraphs of the Codex Plant Guidelines that are not listed can be omitted from consideration.

Description of the recombinant-dna plant

8. Paragraph 22 of the Codex Plant Guideline applies.

Description of the host plant and its use as a food

9. Paragraphs 23, 24 and 25 of the Codex Plant Guideline apply.

[24] This guidance is not intended for a recombinant-DNA plant that was not authorized in an importing country as a result of that country's food safety assessment.

Description of the donor organism(s)

10. Information should be provided on the donor organism(s) and, when appropriate, on other related species. It is particularly important to determine if the donor organism(s) or other closely related members of the family naturally exhibit characteristics of pathogenicity or toxin production, or have other traits that affect human health. The description of the donor organism(s) should include:

A. its usual or common name;
B. scientific name;
C. taxonomic classification;
D. information about the natural history as concerns food safety;
E. information on naturally occurring toxins and allergens; for microorganisms, additional information on pathogenicity and the relationship to known pathogens; and,
F. information on 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)[25].

Description of the genetic modification(s)

11. Paragraphs 27, 28 and 29 of the Codex Plant Guideline apply.

Characterization of the genetic modification(s)

12. Paragraphs 30 and 31 of the Codex Plant Guideline apply.

13. Information should be provided on any expressed substances in the recombinant-DNA plant; this should include: A) the gene product(s) (e.g. a protein or an untranslated RNA); B) the gene product(s)' function; C) the phenotypic description of the new trait(s); D) the level and site of expression in the plant of the expressed gene product(s), and the levels of its metabolites in the edible portions of the plant; and E) where possible, the amount of the target gene product(s) if the function of the expressed sequence(s)/gene(s) is to alter the accumulation of a specific endogenous mRNA or protein.[26]

14. Paragraph 33 of the Codex Plant Guideline applies.

Safety Assessment

Expressed Substances (non-nucleic acid substances)

Assessment of possible toxicity

15. The safety assessment should take into account the chemical nature and function of the newly expressed substance and identify the concentration of the substance in the edible parts of the recombinant-DNA plant, including variations and mean values.[27]

16. Information should be provided to ensure that genes coding for known toxins present in the donor organisms are not transferred to recombinant-DNA plants that do not normally express those toxic characteristics. This assurance is particularly important in cases where a recombinant-DNA plant is processed differently from a donor plant, since conventional food processing techniques associated with the donor organisms may deactivate, degrade or eliminate toxicants.[28]

17. Paragraph 37 of the Codex Plant Guideline applies.

18. In the case of proteins, the assessment of potential toxicity should focus on amino acid sequence similarity between the protein and known protein toxins as well as stability to heat or processing and to degradation in appropriate representative gastric and intestinal model systems. appropriate oral toxicity studies[29] may need to be carried out in cases where the protein present in the food is not similar to proteins that have previously been consumed safely in food, and taking into account its biological function in the plant where known.[30]

19. Paragraphs 39 and 40 of the Codex Plant Guideline apply.

Assessment of possible allergenicity (proteins)

20. Paragraphs 41, 42 and 43 of the Codex Plant Guideline apply.

25 The text of this paragraph was adapted from paragraph 26 of the Codex Plant Guideline.
26 The text of this paragraph was adapted from paragraph 32 of the Codex Plant Guideline.
27 The text of this paragraph was adapted from paragraph 35 of the Codex Plant Guideline.
28 The text of this paragraph was adapted from paragraph 36 of the Codex Plant Guideline.
29 Guidelines for oral toxicity studies have been developed in international fora, for example, the OECD Guidelines for the Testing of Chemicals.
30 The text of this paragraph was adapted from paragraph 38 of the Codex Plant Guideline.

Analyses of Key Toxicants and Allergens

21. Analyses of key toxicants[31] and allergens are important in certain cases of foods from recombinant-DNA plants (e.g., those that are commonly consumed whole and undiluted, such as potatoes, tomatoes, and papaya). Analyses of concentrations of key toxicants and allergens of the recombinant-DNA plant typical of the food should be compared with an equivalent analysis of a conventional counterpart grown and harvested 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. The comparator(s) used in this assessment should ideally be the near isogenic parental line. In practice, this may not be feasible at all times, in which case a line as close as possible should be chosen. The purpose of this comparison 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.[32]

22. The location of trial sites should be representative of the range of environmental conditions under which the plant varieties would be expected to be grown. The number of trial sites should be sufficient to allow accurate assessment of key toxicants and allergens over this range. Similarly, trials should be conducted over a sufficient number of generations to allow adequate exposure to the variety of conditions met in nature. To minimize environmental effects, and to reduce any effect from naturally occurring genotypic variation within a crop variety, each trial site should be replicated. An adequate number of plants should be sampled and the methods of analysis should be sufficiently sensitive and specific to detect variations in key toxicants and allergens.[33]

Evaluation of Metabolites

23. Some recombinant-DNA plants may have been modified in a manner that could result in new or altered levels of various metabolites in the food. In certain cases of foods from recombinant-DNA plants (e.g., those that are commonly consumed whole and undiluted), consideration should be given to the potential for the accumulation of metabolites in the food that would adversely affect human health. Food safety assessment in situations of low level presence of recombinant-DNA material in foods from such plants requires investigation of residue and metabolite levels in the food. 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).[34]

Food Processing

24. The potential effects of food processing, including home preparation, on foods derived from recombinant-DNA plants should also be considered. For example, alterations could occur in the heat stability of an endogenous toxicant. Information should therefore be provided describing the processing conditions used in the production of a food ingredient from the plant. For example, in the case of vegetable oil, information should be provided on the extraction process and any subsequent refining steps.[35]

Potential accumulation of substances significant to human health

25. Some recombinant-DNA plants may exhibit traits (e.g. herbicide tolerance) which may indirectly result in the potential for accumulation of pesticide residues, altered metabolites of such residues, toxic metabolites, contaminants, or other substances which may be relevant to human health. In certain cases of foods from recombinant-DNA plants (e.g. those that are commonly consumed whole and undiluted), the risk assessment should take this potential for accumulation into account. Conventional procedures for establishing the safety of such compounds (e.g. procedures for assessing the human safety of chemicals) should be applied.[36]

Use of antibiotic resistance marker genes

26. Paragraphs 55, 56, 57 and 58 of the Codex Plant Guideline apply.

31 Key toxicants are those toxicologically significant compounds known to be inherently present in the plant, such as those compounds whose toxic potency and level may be significant to health (e.g. solanine in potatoes if the level is increased).

32 The text of this paragraph was adapted from paragraph 44 of the Codex Plant Guideline.

33 The text of this paragraph was adapted from paragraph 45 of the Codex Plant Guideline.

34 The text of this paragraph was adapted from paragraph 46 of the Codex Plant Guideline.

35 The text of this paragraph was adapted from paragraph 47 of the Codex Plant Guideline.

36 The text of this paragraph was adapted from paragraph 54 of the Codex Plant Guideline.

Section 3 – Guidance on data and information sharing

27. In order for Codex Members to use this Annex, it is essential that they have access to requisite data and information.

28. Codex Members should make available to a publicly accessible central database to be maintained by FAO information on recombinant-DNA plants authorized in accordance with the Codex Plant Guideline. This information should be presented in accordance with the following format:

a) name of product applicant;
b) summary of application;
c) country of authorization;
d) date of authorization;
e) scope of authorization;
f) unique identifier;
g) links to the information on the same product in other databases maintained by relevant international organizations, as appropriate;
h) summary of the safety assessment, which should be consistent with the framework of food safety assessment of the Codex Plant Guideline;
i)where detection method protocols and appropriate reference material (non-viable, or in certain circumstances, viable) suitable for low-level situation may be obtained[37]; and
j) contact details of the competent authority(s) responsible for the safety assessment and the product applicant.

29. This process should facilitate rapid access by importing Codex Members to additional information relevant to the assessment of food safety assessment in situations of low-level presence of recombinant-DNA plant material in foods in accordance with this Annex.

30. The authorizing Codex Members should make available complementary information to other Codex Members on its safety assessment in accordance with the Codex Plant Guideline, in conformity with its regulatory/legal framework.

31. The product applicant should provide further information and clarification as necessary to allow the assessment according to this Annex to proceed, as well as a validated protocol for an event-specific or trait-specific detection method suitable for low level situations and appropriate reference materials (non-viable, or in certain circumstances, viable). This is without prejudice to legitimate concerns to safeguard the confidentiality of commercial and industrial information.

32. As appropriate, new scientific information relevant to the conclusions of the food safety assessment conducted in accordance with the Codex Plant Guideline by the authorizing Codex member should be made available ●

34 This information may be provided by the product applicant or in some cases by Codex members.