Food and Agriculture Organization of the United Nations

World Health Organization

Evaluation of Allergenicity of Genetically Modified Foods

Report of a Joint FAO/WHO Expert Consultation on Allergenicity of Foods Derived from Biotechnology

22 – 25 January 2001

Food and Agriculture Organization of the United Nations (FAO)
Rome, Italy

The opinions expressed in this report are those of the participants at the Consultation and do not imply any opinion on the part of FAO and WHO

CONTENTS

1. Introduction

A Joint FAO/WHO Expert Consultation on Foods Derived from Biotechnology was held at the Headquarters of the Food and Agriculture Organization of the United Nations (FAO) in Rome from 22 to 25 January 2001. The Consultation was a follow-up of the Joint FAO/WHO Consultation held in Geneva, Switzerland from 29 May to 2 June 2000 and focused on the question of allergenicity of genetically modified foods. A total of 28 experts, including authors of discussion papers, participated in the Consultation. The complete list of participants is given in Annex 1.

Mr Jacque Vercueil, Director of the Agriculture and Economic Development Analysis Division, Economic and Social Department of FAO, opened the Consultation on behalf of the Directors-General of the World Health Organization (WHO) and FAO. In his statement, Mr Vercueil indicated that allergenicity was one of the most frequently asked questions in connection with the safety of genetically modified foods. It was urgently needed to establish a reliable methodology to assess the allergenicity of new foods produced by the recombinant DNA technique. Applying appropriate risk management measures could reduce the risk of allergenicity of genetically modified foods.

The Consultation elected Dr Dean Metcalfe as Chairperson and Dr Harris Steinman as Vice-Chairperson. Dr Steve Taylor was elected as Rapporteur. The Consultation agreed to base its discussion on the decision tree adapted by the previous FAO/WHO Consultation in 2000 (Annex 3). The Consultation decided to form two working groups to draft the report and to delegate the working groups to elect their chairpersons and rappporteurs: the first working group, considering mainly products created with genes obtained from known allergenic sources (the left-hand side of the existing decision tree, Annex 3) and post-market surveillance, decided that Dr Carsten Bindslev-Jensen be its Chairman and Dr David Hill be its Rapporteur and the second working group, considering mainly products created with genes obtained from sources with no history of allergenicity (the right-hand side of the existing decision tree, Annex 3), decided that Dr Rob Aalberse be its Chairperson and Dr Ricki Helm be its Rapporteur, respectively. The list of working documents is reproduced in Annex 2 to this report. The report entitled "Assessment of Scientific Information Concerning StarLink Corn" (EPA, 2000) was also introduced as an actual case where methodologies under discussion by the Consultation had been applied.

The Consultation further noted the specific questions in documents Biotech 01/02, submitted by the Joint FAO/WHO Secretariat of the Consultation.

2. Background

In 1990 and 1996 FAO and WHO organised joint expert consultations to consider the safety and nutritional aspects of genetically modified foods. The 1990 Consultation regarded biotechnology as a continuum, embracing traditional breeding techniques and modern techniques based on recombinant DNA technologies and concluded that foods from modern biotechnology were inherently not less safe than those from traditional biotechnology (WHO, 1991). The 1996 Consultation recommended that substantial equivalence be an important component in the safety assessment of foods and food ingredients derived from genetically modified plants intended for human consumption (FAO, 1996). The Codex Alimentarius Commission and its relevant subsidiary bodies had reflected the results of the both consultations.

Recognizing the rising concern among the world population about the safety and nutritional aspects of foods derived from biotechnology, the Codex Alimentarius Commission,

at its 23rd Session in 1999, decided to establish an *Ad Hoc* Intergovernmental Task Force on Foods Derived from Biotechnology to develop standards, guidelines or recommendations, as appropriate, for foods derived from biotechnology or traits introduced into foods by biotechnology. The first meeting of the Task Force was held in Japan in March 2000. FAO and WHO expressed their intention to organize a series of scientific expert consultations to support the work of the Task Force.

In June 2000, a Joint FAO/WHO Consultation on Foods Derived from Biotechnology was held in Geneva (WHO, 2000). It addressed the overall safety aspects of foods derived from genetically modified plants and focused on the applicability of substantial equivalence as a general guidance for scientific risk assessment. This Consultation identified specific areas on which further expert consultation was needed and recommended that FAO/WHO should convene an expert consultation on the assessment of allergenicity of genetically modified foods and the novel proteins contained therein as a matter of priority.

The 2000 Consultation adapted a decision-tree (Annex 3) for the evaluation of allergenicity of novel proteins introduced into genetically modified foods. It agreed that the reliability of the risk assessment procedures for allergenicity of genetically modified foods using the decision-tree approach should be further enhanced, including the consideration of additional criteria.

3. Scope

The Consultation was convened to provide FAO, WHO and their Member States with scientific advice in relation to the assessment of allergenicity in genetically modified foods. This would cover in particular:

- General consideration of allergenicity of genetically modified foods
  - consideration of allergenicity specifically relevant to genetically modified foods
- Consideration of the decision-tree approach
  - consideration and possible revision of the decision-tree for the assessment of allergenicity of genetically modified foods developed preliminarily by the June 2000 Joint FAO/WHO Consultation on Foods Derived from Biotechnology.
  - development of standardised procedures for consideration of the use of individual criteria used in the decision tree, with a view to a harmonised application of the decision-tree
  - consideration of the possibility of post market surveillance for inclusion in the decision-tree and technologies supporting the implementation of post market surveillance.
- Specific questions arising in relation to the assessment of allergenicity of genetically modified foods
  - use of databases in the assessment of allergenicity of genetically modified foods
  - use of animal testing
  - other related issues

4. Overview of Food Allergies

Food allergies are adverse reactions to an otherwise harmless food or food component that involves an abnormal response of the body's immune system to specific protein(s) in foods. True food allergies may involve several types of immunological responses (Sampson and Burks, 1996). The most common type of food allergy is mediated by allergen-specific immunoglobulin E (IgE) antibodies[1]. 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 may occur to pollens, mould spores, animal danders, insect venoms and other environmental stimuli as well as foods. IgE-mediated reactions affect perhaps 10-25% of the population in developed countries (Mekori, 1996), although food allergies represent a small fraction of all allergic diseases. Infants and young children are more commonly affected by IgE-mediated food allergies than adults; the prevalence among infants under the age of 3 may be as high as 5-8% (Bock, 1987; Sampson, 1990a; European Commission, 1998).

True food allergies also encompass delayed hypersensitivity reactions whose mechanisms are less clear. Such reactions include cell-mediated reactions that involve sensitised lymphocytes in tissues rather than antibodies (Sampson, 1990b). In cell-mediated reactions, the onset of symptoms occurs more than 8 hours after ingestion of the offending food. The overall prevalence of food-induced, cell-mediated reactions remains uncertain (Burks and Sampson, 1993), but these reactions are well documented in infants. Delayed, food-induced enteropathy has been observed in infants on exposure to milk, soybeans, and less frequently, other proteins. The most common cell-mediated hypersensitivity reaction affecting all age groups of the population is celiac disease, also known as gluten-sensitive enteropathy. Celiac disease affects 1 in every 300 to 3000 individuals in the population depending upon the specific geographic region.

Food allergies are caused by a wide variety of foods. The Codex Committee on Food Labelling established, after considerable debate, a list of the most common allergenic foods associated with IgE-mediated reactions on a worldwide basis that includes peanuts, soybeans, milk, eggs, fish, crustacea, wheat, and tree nuts. This list was presented to the Codex Alimentarius Commission and adopted in 1999 at its 23rd Session. These commonly allergenic foods account for over 90% of all moderate to severe allergic reactions to foods, although an extensive literature search has revealed more that 160 foods associated with sporadic allergic reactions (Hefle et al., 1996). Theoretically, any food that contains protein would be capable of eliciting an allergic reaction, although foods vary widely in their likelihood of provoking allergic sensitisation. In addition to the Codex list, allergic reactions to fresh fruits and vegetables, associated with the oral allergy syndrome (OAS), are also rather common (Ortolani et al., 1988). These foods are not included in the Codex list. The symptoms are typically mild and mostly confined to the oropharyngeal region. Some of the most significant allergens from these foods are unstable to heating and digestion. However, OAS in patients allergic to fruits and vegetables may, in some individuals, be followed by a systemic reaction (Ballmer-Weber et al., 2000). The list established by the Codex Committee on Food Labelling also includes gluten-containing cereals (wheat, rye, barley, oats and spelt) that are implicated in the aetiology of gluten-sensitive enteropathy.

In IgE-mediated food allergies, exposure to a specific food and the proteins contained therein elicits the development of food allergen-specific IgE antibodies. These IgE antibodies

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

attach to the surfaces of mast cells and basophils, thus sensitising the individual to react upon subsequent exposure to the specific food. Thus, to become sensitised, individuals must first be exposed to the food in question. Some food proteins are more likely than others to elicit allergic sensitisation. Very limited information exists on the levels of exposure to a food that are minimally necessary to elicit allergic sensitisation in susceptible individuals. However, infants are much more likely to be sensitised than adults and are possibly sensitised to comparatively low levels of exposure to the offending food. Subsequent exposure of a sensitised individual to the offending food will likely elicit an allergic reaction. The allergen cross-links IgE antibodies on the surfaces of mast cells or basophils triggering the release of various mediators of the allergic reaction. These mediators are released into the tissues and blood, interacting with various receptors that provoke the symptoms characteristic of allergic reactions. The amount of exposure to an ingested, allergenic food protein needed to elicit a discernable reaction in already sensitised and highly sensitive individuals is not precisely known but appears to be in the microgram to low milligram range.

The manifestations of IgE-mediated food allergies range from mild to severe to life-threatening events. Individuals display different thresholds for elicitation of a reaction following ingestion of the offending food. However, the most sensitive food-allergic individuals will experience reactions from exposure to microgram to low milligram quantities or perhaps less of the offending food (limited studies have been conducted on threshold doses so the lowest-observed adverse effect level cannot be deduced precisely for any given allergenic food). Severe reactions can take place after intake of minute amounts of the offending food, and a safe threshold level below which reactions will not occur has not been defined.

Gluten-sensitive enteropathy or celiac disease is a T cell-mediated immunological response triggered by gluten (gliadin) which affects genetically disposed individuals. The active phase of the disease consists of an inflammatory process in the small intestine leading to malabsorption with body wasting, anaemia, diarrhoea, and bone pain along with other symptoms. The disease demands lifelong avoidance of gluten from wheat, rye, barley, and related cereals.

Celiac disease and other enteropathies, although recognized by this Consultation as important medical conditions, were not included in the assessment strategies considered by this Consultation.

Both IgE-mediated food allergies and non-IgE-mediated reactions are treated with specific avoidance diets. Since in both cases, the threshold dose is low and not precisely defined, affected individuals can experience difficulties in the adherence to the avoidance diets.

Almost all food allergens are proteins, although the possibility exists that other food components may act as haptens[2]. While some food allergens have been identified and characterized, many others remain unknown. Many of the known food allergens fall into certain classes of proteins which may aid in the identification of unknown allergens from other sources. 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 thousands of different proteins, relatively few are allergenic. The distribution of these proteins varies in different parts of the plant and can be influenced by environmental factors such as climate and disease stress.

Conventional breeding introduces additional protein diversity into the food supply. However, variations in the protein composition of our diets brought about through conventional

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

crop improvement practices have had little, if any, effect on the allergenic potential of our major foods. In contrast, altered dietary preferences and changes in food manufacturing and food formulation practices can have significant implications for the development of food allergies. For example, allergy to peanut (groundnut) occurs at a significant frequency in North America and Western Europe but not in other countries where peanuts are less commonly eaten. Also, recent food introductions such as kiwi fruit have proven to be additional sources of food allergens. With respect to food formulations, the wider distribution of certain ethnic foods, such as those containing sesame seeds, may contribute to increases in allergic sensitivity to certain foods. These observations provide confidence that there are not a large number of potential allergens in the food supply, but show that new allergenic foods are sometimes introduced into the marketplace.

Because of the above, a clear need exists to pay particular attention to allergenicity when assessing the safety of foods produced through genetic modification. In the assessment of the allergenicity of genetically modified foods, the characteristics of the novel gene products (proteins) must be evaluated in light of their similarities to known food and environmental allergens. In addition, if an examination of the genetically modified food in comparison to its conventional counterpart reveals the presence of any unintended, new proteins resulting from the transformation events, these unintended, new proteins should also be assessed for their possible allergenicity using a similar approach.

5. Decision Tree Approach to the Evaluation of the Allergenicity of Genetically Modified Foods

5.1. Introduction

In 1996, the International Food Biotechnology Council and the Allergy and Immunology Institute of the International Life Sciences Institute (IFBC/ILSI) presented a decision-tree approach to the evaluation of the potential allergenicity of the novel gene products (proteins) in genetically modified foods (Metcalfe et al., 1996). This allergy assessment strategy has been widely adopted by the agricultural biotechnology industry. It is a strategy which focuses on the source of the gene, the sequence homology of the newly introduced protein to known allergens, the immunochemical binding of the newly introduced protein with IgE from the blood serum of individuals with known allergies to the transferred genetic material, and the physicochemical properties of the newly introduced protein (Metcalfe et al., 1996; Taylor, 1997).

In the 1996 Joint FAO/WHO Consultation on Biotechnology and Food Safety, the issue of allergenicity of genetically modified foods was specifically addressed for the first time. An assessment approach similar to that developed by IFBC/ILSI was advocated that included the following criteria: source of the transferred genetic material, molecular weight, sequence homology, heat and processing stability, effect of pH and/or gastric juices (digestive stability), and prevalence in foods. The 1996 Consultation concluded that "a rational scientific approach to the assessment of the allergenicity of genetically modified organisms can and should be undertaken" as part of the overall safety assessment approach. Furthermore, the 1996 Consultation made several recommendations relative to allergenicity of genetically modified foods:

- The transfer of genes from commonly allergenic foods should be discouraged unless it is documented that the gene transferred does not code for an allergen.

- Foods found to contain an allergen transferred from the organism which provided the DNA should not be considered for marketing approval unless such products can be clearly identified in the marketplace and this identity will not be lost through distribution and processing. Further, that labelling approaches may not be practical in these situations, and that particular problems exist for consumers who cannot read, or who may not be provided with labels.
- Involved organizations should consider the appropriateness of, and/or actions to take, in respect to foods containing new protein(s) that are determined to have the characteristics of an allergen, even though no patient population is known to exist which has an allergy to this gene product.
- The identification of food allergens and the characteristics of these allergens that define their immunogenicity be encouraged.

In the 2000 Joint FAO/WHO Consultation on Safety Aspects of Genetically Modified Foods of Plant Origin, the issue of the allergenicity of genetically modified foods was specifically addressed again. The IFBC/ILSI decision-tree approach was adapted, with minor changes, for the evaluation of novel proteins introduced into genetically modified foods (Annex 3). The said Consultation concluded "that if a genetically modified food contains the product of a gene from a source with known allergenic effects, the gene product should be assumed to be allergenic unless proven otherwise. The transfer of genes from commonly allergenic foods should be discouraged unless it can be documented that the gene transferred does not code for an allergen. The novel proteins introduced into genetically modified food should be evaluated for allergenicity on the basis of the decision-tree shown in Annex 3." The 2000 Consultation noted that the IFBC/ILSI decision tree as adapted by FAO/WHO in Annex 3 had received some criticism related to certain of the criteria involved in the decision tree. The 2000 Consultation further concluded, "additional criteria should be considered for the addition to the decision-tree approach when the source of the genetic material is not known to be allergenic. The level and site of expression of the novel protein and the functional properties of the novel protein would be two such criteria."

The 2000 Joint FAO/WHO Consultation recommended that "WHO/FAO should be encouraged to convene an Expert Consultation on the assessment of the allergenicity of genetically modified foods and the novel proteins contained therein. The Consultation should focus on the development of an improved decision-tree approach for the assessment of the allergenicity of genetically modified foods and on the standardization/validation of specific criteria, such as optimal methods for assessment of digestive stability." With this background, the current consultation undertook efforts to develop an improved decision-tree approach using as a start, the existing IFBC/ILSI decision tree as adapted by the 2000 FAO/WHO Consultation (Annex 3) .

5.2. The FAO/WHO 2001 Decision Tree

After consideration of the current status of scientific information and extensive discussion, the Consultation developed a new decision tree (Annex 4) that will be referred to throughout the remainder of this report as the FAO/WHO 2001 decision tree. This new decision tree builds upon previous approaches to examining allergenicity but also encompasses several additional strategies.

5.3. Food containing a gene derived from a source known to be allergenic

When the expressed protein comes from a source known to be allergenic, the analysis presented in the FAO/WHO 2001 decision tree focuses on both sequence homology and subsequent assessment of potential allergenicity of the expressed protein with sera of patients allergic to the source material (Annex 4). Sequence homology is the initial step to be performed. Criteria for a positive outcome in the analysis of sequence homology are reviewed in Section 6.1. When sequence homology to a known allergen is demonstrated, the product is considered allergenic, and no further testing is typically undertaken. If no sequence homology to a known allergen is demonstrated, specific serum screening for the expressed protein is undertaken. These investigations focus on assessment of the possible allergenicity of the expressed protein using sera from patients allergic to the source material (Section 6.2). These patients should be carefully defined according to international guidelines. If the patients donating sera have a low level of sensitisation, the usefulness of those sera in exhibiting reactivity to the expressed protein may be compromised. Therefore, it is suggested to include only patients with a level of sensitisation to the allergen source of more than 10 kIU/L of specific IgE.

In contrast to previous decision-tree strategies, the FAO/WHO 2001 decision tree makes no distinction between commonly and less commonly allergenic source materials with respect to specific serum screening. Thus, specific serum screening is undertaken irrespective of the relative frequency of allergy to the source material in question, provided sera are available (Section 6.2). Insufficient information exists in the literature supporting an increased risk of a severe reaction for patients with hypersensitivity to commonly allergenic foods as opposed to less commonly allergenic foods.

The degree of confidence in the results of the specific serum screening will depend upon the number of sera that are available for analysis. To achieve 95 % certainty that a major allergen (a major allergen is defined as one to which more than 50 % of individuals sensitive to that substance react in IgE-specific immunoassays) from the source material has not been transferred, a negative result must be obtained with at least 6 relevant sera. To achieve 99 % certainty that a major allergen from the source material has not been transferred, a negative result must be obtained with at least 8 relevant sera. To achieve 99.9 % certainty that a major allergen from the source material has not been transferred, a negative result must be obtained with at least 14 relevant sera. Furthermore, by using 17 relevant test sera, a 95 % probability exists of detecting a minor allergen (a minor allergen is defined as one to which less than 50 % of individuals sensitive to that substance react in IgE-specific immunoassays) from the source to which at least 20 % of the affected population are reactive. By using 24 relevant sera, a 99 % probability exists of detecting a minor allergen from the source to which at least 20 % of the affected population are reactive. An argument can be made for using fewer sera if relevant sera are not available, but this modified approach carries the risk of a false negative outcome. However, the use of larger numbers of sera is advocated, whenever possible, to increase the confidence associated with negative immunoassay results as described above. The Consultation also recognizes that the use of a smaller number of very well documented, high quality sera may be preferable to the use of larger numbers of lesser-quality sera. The in vitro method applied should be a validated assay measuring specific IgE (Section 6.2).

Any positive outcome defines the product as likely allergenic, and will normally lead to discontinuation of product development. A negative outcome of the specific serum screening prompts further analysis using targeted serum screening (Section 6.3), pepsin resistance (Section 6.4) and animal models (Section 6.5) (see Annex 4). Additionally, in vivo/ex vivo[3] testing in

[3] "in vivo (using allergic human subjects)/ex vivo (using cells or tissue culture from allergic human subjects)

allergic patients may also be appropriate in circumstances where confirmation of positive results in the specific serum screening is wished; or where a negative outcome of appropriate in vivo/ex vivo testing would be more convincing than a positive outcome of the specific serum screening provided that well documented allergic subjects were used in the in vivo/ex vivo testing. The ex vivo/in vivo methods include skin prick testing (Bruijnzeel-Koomen et al, 1995), basophil histamine release (Bindslev-Jensen and Poulsen, 1996) and oral challenge (Bock et al, 1988; Bruijnzeel-Koomen et al, 1995). It is anticipated that these procedures will require approval from Ethics Committees (Internal Review Boards). Therefore, the FAO/WHO 2001 decision tree does not include human in vivo testing as a mandatory tool, but in vivo testing may be considered in selected cases.

An equivocal outcome of the specific serum screening would lead to further analysis using targeted serum screening, pepsin resistance or animal models (see Annex 4). Again, ex vivo/in vivo testing involving patients allergic to the source material may also be considered.

The FAO/WHO 2001 decision tree is not applicable to the evaluation of foods where gene products are down-regulated for hypoallergenic purposes. In such cases, in vivo testing including skin prick testing, open challenges, and double-blind, placebo-controlled food challenges would be required.

5.4. Food containing a gene derived from a source not known to be allergenic

When the expressed protein comes from a source that is not known to be allergenic, the FAO/WHO 2001 decision tree focuses on (1) sequence homology to known allergens (food and environmental), (2) targeted serum screening for cross-reactivity with sera from patients allergic to materials that are broadly related to the source material for the gene, (3) pepsin resistance and (4) immunogenicity testing in animal models (Annex 4). In this situation the search for homologous allergens is based on two procedures.

The first step is a database search for an allergen with a homologous amino acid sequence, according to the principles described in Section 6.1. If this search reveals a level of homology with a known allergen that suggests a potential for cross-reactivity, the expressed protein is considered to be an allergenic risk. No further evaluation for allergenicity would typically be necessary.

The second step is conducted if no such homologous protein is found. In such cases, cross-reactivity is tested with a panel of serum samples that contain high levels of IgE antibodies with a specificity that is broadly related to the gene source (Section 6.3). For this "targeted serum screen", 6 groups of source organisms are distinguished: yeast/moulds, monocots, dicots, invertebrates, vertebrates and "others". A panel of 50 serum samples with high levels of IgE to allergens in the relevant group is used to search for IgE antibodies that are cross-reactive with the expressed protein. If a positive reaction is obtained with one of these sera, the expressed protein is considered to be an allergenic risk and further evaluation for allergenicity would typically not be necessary. If a gene were obtained from a bacterial source, no targeted serum screening would be possible, since no normal population of individuals are known to be sensitised (IgE mediated) to bacterial proteins.

When a positive outcome is obtained in targeted serum screening, further evaluation using in vivo/ex vivo approaches as described in Section 5.3 may be conducted if desired to seek confirmation of the results of the targeted serum screening. If the results obtained with in vivo/ex vivo testing differ from those obtained with targeted serum screening, these results would be more convincing than a positive outcome in the targeted serum screening provided that appropriate, well documented allergic subjects were used in the in vivo/ex vivo testing.

If no cross-reactive serum is found, the protein is analysed for pepsin resistance and for evidence of immunogenicity in appropriate animal models according to the protocols provided in Sections 6.4 and 6.5.

5.5. Post marketing surveillance

The Consultation acknowledges that the pre-market allergenicity assessment of the genetically modified food gives a satisfactory safety assurance. However, it is recognised that due to the wide genetic variability in the human population and different geographical dietary intake, further evaluation for adverse effects of the genetically modified food should be considered once the product has reached the market. This could provide additional safety assurance.

Ideally, a notifying, self-reporting system for any adverse health effects, both for consumers and for employees in the food production industry should be put in place. Reported data should be validated with respect to:

- the clinical outcome in relation to allergenicity
- the causality between the reported adverse effect and the specific genetically modified food/food ingredient exposure

These validated data should be recorded, consolidated and published. Such a system could benefit from experiences of existing national surveillance systems (e.g. disease control centres, poisoning centres).

However, the feasibility of post-marketing surveillance systems should be further explored, since there are number of problems to be addressed, including:

- traceability and labelling of the genetically modified food/food ingredient
- lack of background data on prevalence and incidence in food related allergies
- existence of many confounding food and non-food related factors
- changes in diets over time
- lack of trained experts and infrastructure, especially in developing country settings

5.6. Other Criteria that were Considered

5.6.1. Level of expression

Highly allergenic proteins are often expressed at relatively high levels. However, allergens can sensitize susceptible individuals at less than milligram levels, possibly at less than microgram levels (Sorva et al., 1994; Jarvinen et al., 1999). The elicitation of objective symptoms in already sensitized individuals can also occur at low levels of exposure, but has not been documented below 500 micrograms (Rance and Dutau, 1997; Hourihane et al., 1997). It is therefore not possible to define a level of expression below which a protein can be considered safe from the allergenicity point of view. Thus, level of expression cannot yet be incorporated into the assessment of the allergenicity of genetically modified foods.

5.6.2. Unintended effects

In achieving the objective of conferring a specific target trait (intended effect) to the host organism by the insertion of DNA sequences, additional traits could, theoretically, be acquired or existing traits lost or enhanced (unintended effects). Unintended effects may be due to factors such as random insertion events, which might result in disruption of existing genes and

modification of protein expression. While unintended effects are not specific to the use of recombinant DNA techniques, any such effects should be identified to the maximum extent possible and their impact upon the allergenicity of the genetically modified food should be assessed.

With respect to allergenicity, two types of unintended effects could be envisioned. First, the gene insert may activate or suppress existing host genes in an inordinate fashion leading to either over-expression or under-expression of specific proteins. If the host plant contains known allergenic proteins, then the possibility that the levels of these allergens has been elevated should be considered as part of the safety evaluation process. Secondly, if evidence is obtained from comparison of the genetically modified food to its conventional counterpart that the insertion of the gene creates additional new proteins, then these proteins should be evaluated for their potential allergenicity using the approach described herein.

6. Standardization of Methodologies

6.1. Sequence Homology as Derived from Allergen Databases

The commonly used protein databases (PIR, SwissProt and TrEMBL) contain the amino acid sequences of most allergens for which this information is known. However, these databases are currently not fully up-to-date. A specialized allergen database is under construction.

Suggested procedure on how to determine the percent amino acid identity between the expressed protein and known allergens.

Step 1: *obtain the amino acids sequences of all allergens in the protein databases (for SwissProt and TrEMBL: see http://expasy.ch/tools; for PIR see http://www-nbrf.georgetown.edu/pirwww) in FASTA-format (using the amino acids from the mature proteins only, disregarding the leader sequences, if any). Let this be data set (1).*

Step 2: *prepare a complete set of 80-amino acid length sequences derived from the expressed protein (again disregarding the leader sequence, if any). Let this be data set (2).*

Step 3: *go to EMBL internet address: http://www2.ebi.ac.uk and compare each of the sequences of the data set (2) with all sequences of data set (1), using the FASTA program on the web site for alignment with the default settings for gap penalty and width.*

Cross-reactivity between the expressed protein and a known allergen (as can be found in the protein databases) has to be considered when there is:

1) more than 35 % identity in the amino acid sequence of the expressed protein (i.e. without the leader sequence, if any), using a window of 80 amino acids and a suitable gap penalty (using Clustal-type alignment programs or equivalent alignment programs)

or:

2) identity of 6 contiguous amino acids.

If any of the identity scores equals or exceeds 35 %, this is considered to indicate significant homology within the context of this assessment approach. The use of amino acid

sequence homologies to identify prospective cross-reacting allergens in genetically modified foods has been discussed in more detail elsewhere (Gendel, 1998a; Gendel, 1998b).

Structural similarity with known allergens may still be important if significant amino acid identity is found, but it is below 35 %. In this case significant cross-reactivity is unlikely. However, some families of structurally related proteins are known to contain several allergens. Some examples are:

- lipocalins
- non-specific lipid transfer proteins
- napins (2S albumins from seeds)
- parvalbumins.

If the expressed protein belongs to such a family, it may be considered to have a higher probability to be an allergenic protein.

Functional similarity without structural similarity is unlikely to result in significant cross-reactivity. For example, protease inhibitors that belong to distinct protein families are not known to be cross-reactive. Similarly, proteins belonging to structurally unrelated classes of pathogenesis-related proteins (PR-proteins) are not known to be cross-reactive.

Since identity of 6 contiguous amino acids has an appreciable risk of occurring by chance, verification of potential crossreactivity is warranted when criterion (1) is negative, but criterion (2) is positive. In this situation suitable antibodies (from human or animal source) have to be tested to substantiate the potential for crossreactivity.

6.2. Specific serum screening

In the evaluation of the reactivity of IgE antibodies in the sera of patients with known allergies to relevant source materials, an appropriate in vitro method should be applied. A variety of well validated immunoassays are available for this purpose. The Consultation agrees that any of these tests can be used.

In addition to the precautions cited earlier with respect to selection of suitable sera for such screening, the importance of glycosylation and glycan epitopes must also be considered. Proteins to be expressed in plant hosts may be posttranslationally modified, which may have an impact on their allergenic potential. The effects of glycosylation are particularly relevant to consider, because:

1. The degree of glycosylation may affect the susceptibility of the protein to processing and proteolysis;
2. Glycosylation may alter the epitope structure, either by shielding part of the protein surface (particularly if the glycosylation is extensive), or by introducing glycan epitopes. Glycan epitopes are known to be highly cross-reactive

Glycans may be attached either via an N-link or via an O-link. N-linked sites can be predicted with some accuracy, but the prediction of sites for O-glycosylation is still unreliable.

Cross-reactivity of IgE antibodies to glycan epitopes is important not so much because of their potential contribution to allergic symptomatology (which may be minimal in many cases), but because the structure of the protein part of these glycoproteins is in this situation largely irrelevant: all proteins with these glycan structures will be cross-reactive. When target glycoproteins are screened for cross-reactivity, it is important to make a clear distinction between IgE antibodies to the glycan part on the one hand and IgE antibodies to the protein part

on the other hand. In general, it is advisable to select serum samples without IgE antibodies to glycans, absorb out such IgE antibodies with irrelevant glycoproteins obtained from the same host, or perform such tests with non-glycosylated variants, e.g. expressed in a bacterial host.

Information on glycan epitopes in relation to allergy is largely based on work with plant glycoproteins and invertebrate glycoproteins. Less is known about glycoproteins of eukaryotic microorganisms such as yeast. However, it is likely that similar precautions may need to be taken.

6.3. Targeted serum screening

When no sequence homology has been found between the expressed protein and an allergen, this does not mean that there is no such homologous allergen. It may be due to a lack of information on the relevant allergen. Random screening of serum samples from the allergic population is unlikely to be rewarding. However, some more targeted approach may, in some situations, be more appropriate.

- If the recombinant protein is derived from a monocot, it is proposed to test serum samples from patients with high levels of IgE antibodies to monocot allergens such as grass and rice.
- If the recombinant protein is derived from a dicot, it is proposed to test serum samples from patients with high levels of IgE antibodies to dicot allergens such as tree pollen, weed pollen, celery, peanuts, tree nuts and latex.
- If the allergen is derived from a mould, it is proposed to test serum samples from patients with high levels of IgE antibodies to moulds, yeast and fungi, such as Alternaria or Cladosporium, and of patients with aspergillosis or Trichophyton sensitivity.
- If the allergen is derived from an invertebrate, it is proposed to test serum samples from patients with high levels of IgE antibodies to invertebrates such as mites, cockroach, shrimp, chironimids or silk.
- If the allergen is derived from a vertebrate, it is proposed to test serum samples from patients with high levels of IgE antibodies to mammalian pets, laboratory animals, cow's milk, fish, chicken egg white and chicken egg yolk/serum proteins.
- If the allergen is derived from another source, e.g. a bacterium, no general screen using targeted sera is currently available.

The use of large serum pools (> 5 sera) is discouraged, because this will dilute any cross-reactive antibody present. For maximal sensitivity, individual sera should be tested.

Typically, a screen with 25 individual serum samples with high levels of IgE to the selected group of airborne allergens and (if applicable) 25 with IgE to the selected group of food allergens would be used.

6.4. Pepsin Resistance

Purified or enriched expressed protein (non-heated and non-processed) should be subjected to pepsin degradation conditions using Standard Operating Procedures and Good Laboratory Practices (SOP/GLP). In addition, the expressed protein should be assessed in its principal edible form under identical pepsin degradation conditions to those used to examine the expressed protein. Both known non-allergenic (soybean lipoxygenase, potato acid phosphatase or equivalent) and allergenic (milk beta lactoglobulin, soybean trypsin inhibitor or equivalent) food proteins should be included as comparators to determine the relative degree of the expressed proteins pepsin resistance. The protein concentrations should be assessed using a colorimetric

assay (e.g., Bicinchoninic acid assay (BCA), Bradford Protein Assay, or equivalent protein assay) with bovine serum albumin (BSA) as a standard. Pepsin proteolytic activity should be assessed (Ryle). Enzyme/protein mixtures should be prepared using 500 µg of protein in 200 µL of 0.32% pepsin (w/v) in 30 mM/L NaCl, pH 2.0, and maintained in a shaking 37 C water bath for 60 minutes. Individual 500 microgram aliquots of pepsin/protein solution should be exposed for periods of 0, 15, 30 seconds and 1, 2, 4, 8, 15, and 60 minutes, at which time each aliquot should be neutralised with an appropriate buffer. Neutralised protein solutions should be mixed with SDS-PAGE sample loading buffer with and without reducing agent (DTT or 2-ME) and heated for 5 minutes at 90°C. Samples containing 5µg/cm gel of protein should be evaluated using 10-20% gradient Tricine SDS-PAGE gels or equivalent gel system under both non-reducing and reducing electrophoretic conditions. Protein in the gels should be visualised by silver or colloidal gold staining procedures. Evidence of intact expressed protein and/or intact fragments greater than 3.5 kDa would suggest a potential allergenic protein. Evidence of protein fragments less than 3.5 kDa would not necessarily raise issues of protein allergenicity and the data should be taken into consideration with other decision tree criteria. For detection of expressed protein in an edible food source, a polyclonal IgG immunoblot analysis should be performed according to the laboratory procedures. The immunoblot analysis should be compared to the silver or colloidal gold stained SDS-PAGE gel and reflect the stained pattern of the expressed protein run under identical conditions.

The investigator should be aware of and consider the following precautions. Edible food sources may contain protease inhibitors or other substances that may promote or reduce protein degradation. Resulting fragments may not be reactive with the polyclonal IgG antibody source. Finally, there is no absolute certainty that pepsin resistance or complete degradation of a protein will predict the allergenicity of novel proteins and must be taken into consideration with other decision tree criteria. Although the present pepsin resistance protocol is strongly recommended, it is recognized that other enzyme susceptibility protocols exist. Alternative protocols may be used for which adequate justification is provided. The producer is expected to take these results into consideration in combination with other decision tree criteria.

6.5. Animal Models

For additional assessment of the potential allergenicity of expressed proteins, informative data can be generated using animal models in development. A number of animal models may be considered to assess on a relative scale the potential allergenicity using oral sensitisation routes with the Brown Norway rat model (Knippels et al., 1998) or intraperitoneal administration in murine models (Dearman et al 2000) or other relevant animal models. Results should be presented in characteristic Th1/Th2 antibody (isotype) profiles for assessing the potential immunogenic/allergenic activity. The different routes of administration in animal models (oral versus intraperitoneal) may not give the same results. Therefore, selection of one route of administration is not meant to exclude other routes of sensitisation. It is recommended to consider the results from two sensitisation routes in the same or different animal species.

It is recommended that the potential allergenicity of the expressed protein be ranked against well known strong and weak food allergens and non-allergenic proteins in the animal model. As additional information becomes available with respect to animal models, protocols may need to be modified to give optimal conditions for assessing protein allergenicity.

Although the present animal models provide additional information on potential allergenicity of novel proteins, they do not reflect all aspects of IgE-mediated food allergies in humans.

7. Conclusions

1. The Consultation agreed that the safety assessment of foods derived from biotechnology requires an integrated and stepwise, case-by-case approach, and that this method also be applied to the evaluation of the allergenicity of food derived from biotechnology.
2. The Consultation emphasized that all foods derived from biotechnology must be assessed for allergenic potential.
3. The original decision tree from the FAO/WHO 2000 Consultation served as a basis for this consultation. The Consultation concurred that this decision tree be modified as a consequence of more recent research and which is reflected in the FAO/WHO 2001 decision tree.
4. When the expressed protein is derived from a source with known allergenicity, the FAO/WHO 2001 decision tree proposes that the initial investigation be analysis of sequence homology to known allergens in the source. If this is negative, the next step will be investigations on possible IgE binding using immunoassays and may also include investigations in vivo in patients allergic to the source food.
5. When the expressed protein is derived from a source with no known allergenicity, the FAO/WHO 2001 decision tree proposes that the initial investigation would also be analysis of sequence homology to known allergens from food and environmental sources. If positive matches are found with known allergens, then the protein is considered likely allergenic. If no significant sequence homology is identified, then targeted serum screening is conducted with serum samples that contain high levels of IgE antibodies with a specificity that is broadly related to the gene source. If the targeted serum screening is positive, then the protein is considered likely allergenic. If the targeted serum screening is negative, then pepsin resistance of the expressed protein and the immunogenicity of the expressed protein in suitable animal models are to be assessed to determine the likelihood that the protein will be allergenic.
6. The Consultation agreed that the FAO/WHO 2001 decision tree is not applicable to the evaluation of foods where hypo-allergenicity has been induced by down-regulation of genes.
7. The Consultation was of the opinion that an evaluation of proteins for sequence homology with sufficient sensitivity and specificity to detect potential cross-reactivity is an important part of the process for the assessment of the allergenicity of the expressed protein.
8. The Consultation agreed that further studies would be required to determine the amount of allergen that sensitises and elicits allergic events.
9. The Consultation recognized the need to constantly update allergen databases.
10. The Consultation concluded that animal models have not been evaluated for all food allergens but there is sufficient scientific evidence that using these models will contribute valuable information regarding the allergenicity of foods derived from biotechnology.
11. The Consultation agreed that pepsin susceptibility is a relevant parameter for the identification of potential allergens and that the protocol described is not intended to mimic the physiologic conditions of gastric digestion.

12. The use of human in vivo methods to evaluate the allergenicity of foods derived from biotechnology may in many circumstances raise ethical issues and their use will have to be considered on a case-by-case basis.

13. Post-market surveillance is a valuable tool in the monitoring of adverse effects and long-term sequelae of foods derived from biotechnology and the Consultation recognized that the feasibility of certain aspects of its implementation would need further investigation.

14. The Consultation accepted that the FAO/WHO 2001 decision tree and its accompanying clarifying text will require modification in the future as a result of the rapidly expanding scientific base in the allergy and biotechnology fields but that this decision tree is appropriate based on our present knowledge.

8. Recommendations

1. The Consultation recommends that the FAO/WHO 2001 decision tree be used for determining allergenicity of foods derived from biotechnology.
2. The Consultation recommends that FAO and WHO should endeavour to update the decision tree as and when required.
3. The identification of food allergens and the characteristics of these allergens that define their immunogenicity are encouraged.
4. Protein and gene databases required for the assessment of allergenicity of foods derived from biotechnology should be frequently updated and maintained.
5. Further research is needed on the development and validation of suitable animal models and procedures for the assessment of allergenicity of foods derived from biotechnology.
6. The Consultation recommends that the possibility of implementing post-marketing surveillance should be further studied.
7. The Consultation recommends that FAO and WHO provide technical support to member countries to strengthen their capacity and infrastructure to enable those countries to undertake the evaluation of the allergenicity of foods derived from biotechnology.
8. The Consultation recommends to FAO and WHO the establishment of a coordination network to promote and strengthen the interaction between experts to improve standard operating procedures, good laboratory practices and good clinical practice to facilitate the evaluation of the allergenicity of foods derived from biotechnology.

9. List of Abbreviations

BCA: Bicinchoninic acid assay

BSA: Bovine Serum Albumin

DNA: Deoxyribonucleic acid

DTT: Dithiothreitol

EAACI: European Academy of Allergology and Clinical Immunology

FAO: Food and Agriculture Organization of the United Nations

GM: Genetically Modified

GLP: Good Laboratory Practices

IFBC: International Food Biotechnology Council

Ig: Immunoglobulin

IgE: Immunoglobulin E

IgG: Immunoglobulin G

ILSI: International Life Science Institute

kIU/L: Kilointernational Units/Litre

kDa: Kilodalton

ME: Mercaptoethanole

OAS: Oral allergy syndrome

PR-proteins: Pathogenesis-Related proteins

Th1: T-helper lymphocytes 1, which assist the differentiation of cytotoxic cells and also activate macrophages, which after activation play a role as effectors of the immune response.

Th2: T-helper lymphocytes 2, which are mainly involved in the amplification of B lymphocyte responses.

SCOOP/NUTR/REPORT/2: Scientific Cooperation Programme/Nutrition/Report/2

SDS-PAGE: Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

SOP: Standard Operating Procedures

WHO: World Health Organization

10. References

1. Ballmer-Weber BK, Vieths S, Luttkopf D, Heuschmann P, Wuthrich B (2000): *Celery allergy confirmed by double-blind, placebo-controlled food challenge: a clinical study in 32 subjects with a history of adverse reactions to celery root.* J Allergy Clin Immunol, 106(2): 373-8
2. Bindslev-Jensen C, Poulsen LK (1996): *In vitro diagnostic tests.* Chapter 7 In: Sampson HA, Simons E, Metcalfe DD: Food Allergy 2nd edition, Blackwell Scientific Publications: 137-150
3. Bock SA (1987): *Prospective appraisal of complaints of adverse reactions to foods in children during the first three years of life*. Paediatrics, 79: 683-688
4. Bock SA, Sampson HA, Atkins FM, Zeiger RS, Lehrer S, Sachs M, Bush RK, Metcalfe DD (1988): *Double-blind, placebo-controlled food challenge (DBPCFC) as an office procedure: a manual.* J Allergy Clin Immunol, 82(6): 986-97
5. Burks AW, Sampson H (1993): *Food allergies in children.* Current Problems in Paediatrics 23: 230-252
6. Bruijnzeel-Koomen C, Ortolani C, Aas K, Bindslev-Jensen C, Björksten B, Moneret-Vautrin D, Wütrich B (1995): *Adverse reactions to food. Position Paper.* Allergy, 50: 623-635
7. Dearman RJ, Caddick H, Basketter DA, Kimber I (2000): *Divergent antibody isotope responses induced in mice by systemic exposure to proteins: a comparison of ovalbumin with bovine serum albumin*. Food Chem Toxicol, 38: 351-360
8. EPA (2000): *A Set of Scientific Issues Being Considered by the Environmental Protection Agency Regarding Assessment of Scientific Information Concerning StarLink Corm.* FIFRA Scientific Advisory Panel Meeting, Environmental Protection Agency of the United States
9. European Commission (1998): *Consideration of the Epidemiological Basis for appropriate Measures for the Protection of the Public Heath in Respect of Food Allergy*, SCOOP/NUTR/REPORT/2, European Commission, Brussels
10. FAO (1996): *Biotechnology and food safety, Report of a joint FAO/WHO consultation.* FAO Food and Nutrition Paper **61**, Food and Agriculture Organisation of the United Nations, Rome
11. Gendel SM (1998a): *Sequence databases for assessing the potential allergenicity of proteins used in transgenic foods*. Adv. Food Nutr. Res. 42: 63-92
12. Gendel SM (1998b): *The use of amino acid sequence alignments to assess potential allergenicity of proteins used in genetically modified foods*: Adv. Food Nutr. Res. 42: 45-62
13. Hefle SL, Nordlee JA, Taylor SL (1996): *Allergenic foods*. Critical Reviews in Food Science and Nutrition 36: S69-S89
14. Hourihane JO'B, Kilburn SA, Nordlee J, Hefle S, Taylor SL, Warner JO (1997): *An evaluation of the sensitivity of subjects with peanut allergy to very low doses of peanut protein: a randomized, double-blind, placebo-controlled food challenge study.* J. Allergy Clin. Immunol, 100: 596-600
15. Jarvinen KM, Makinen-Kiljunen S, Suomalainen H (1999): *Cow's milk challenge through human milk evokes immune responses in infants with cow's milk allergy.* J. Pediatr, 135:506-512

16. Knippels et al. (1998): *Oral sensitization to food proteins: a Brown Norway rat model.* Clin Exp Allergy, 28: 368-375
17. Mekori YA (1996): *Introduction to allergic diseases*. Critical Reviews in Food Science and Nutrition 36: S1-S18
18. Metcalfe DD, Astwood JD, Townsend R, Sampson HA, Taylor SL, Fuchs RL (1996): *Assessment of the allergenic potential of foods derived from genetically engineered crop plants*. Critical Reviews in Food Science and Nutrition 36: S165-S186
19. Ortolani C, Ispano M, Pastorello E, Bigi A, Ansaloni R (1988)*: The oral allergy syndrome.* Ann Allergy 61(6 Pt 2): 47-52
20. Rance F, Dutau G (1997): *Labial food challenge in children with food allergy.* Pediatr Allergy Immunol 8: 41-44
21. Sampson HA (1990a): *Food Allergy*. Current Opinion in Immunology 2: 542-547
22. Sampson HA (1990b): *Immunologic mechanisms in adverse reactions to foods*. Immunology and Allergy Clinics of North America 11: 701-706
23. Sampson HA, Burks AW (1996): *Mechanisms of food allergy*. Annual Review of Nutrition 16: 161-177
24. Sorva R, Makinien-Kiljunen S, Juntunen-Backman K (1994): *B-Lactoglobulin secretion in human milk varies widely after cow's milk ingestion in mothers of infants with cow's milk allergy*: J Allergy Clin Immunol 93: 787-792
25. Taylor, S. L. (1997): *Food from genetically modified organisms and potential for food allergy.* Environ Toxicol Pharmacol 4: 121-126
26. WHO (1991): *Strategies for assessing the safety of foods produced by biotechnology.* Report of a Joint FAO/WHO Consultation, World Health Organization, Geneva
27. WHO (2000): *Safety aspects of genetically modified foods of plant origin.* Report of a Joint FAO/WHO Expert Consultation, World Health Organization, Geneva

Annex 1

List of Participants

EXPERTS

CALDAS, Luiz Q de A, Director, Poison Control Center, Antonio Pedro University Hospital, Fluminense Federal University, Rua Marques do Paraná, 353 – 3º. andar – Centro-Niterói – Rio de Janeiro, Brazil
Tel: +55-21-717-0148
Fax: +55-21-717-4459/717-0521
E-mail: ccilqac@vm.uff.br

EGWANG, Thomas, Med Biotech Laboratories, P. O. Box 9364, Kampala, Uganda
Tel: +256-41-268251/266445
Fax: +256-41-268251
E-mail: egwang@imul.com

KUIPER, Harry A., Head, Department of Food Safety and Health, State Institute for Quality Control of Agricultural Products (RIKILT), Wageningen UR, PO Box 230, NL-6700 AE Wageningen, The Netherlands
Tel: + 31 317 475 463
Fax: +31 317 417 717
E-mail: h.a.kuiper@rikilt.wag-ur.nl

LEE, Sang IL, Professor, SungKyunKwan University, School of Medicine, Department of Pediatrics, Samsung Seoul Hospital, #50 Ilwon-dong, Kangnam-ku, Seoul, Republic of Korea
Tel: +82-2-3410-3521
Fax: +82-2-3410-0043
E-mail: silee@smc.samsung.co.kr

MALMHEDEN YMAN, Ingrid, Senior chemist, National Food Administration, Research & Development, P.O.Box 622, SE-751 26 Uppsala, SWEDEN
Tel + 46 18 17 56 82
Fax + 46 18 10 58 48
E-mail: iyma@slv.se

METCALFE, Dean, Chief, Laboratory of Allergic Diseases,
National Institute of Allergy and Infectious Diseases, National Institutes of Health
Building 10, Room 11C205, 10 Center Dr. MSC 1881, Bethesda, MD 20892-1881 USA
(Chairperson)
Tel: +1-301-496-2165
Fax: +1-301-480-8384

MOUSSA, Amel, Etablissement Publique de Santé, Charle Nicolle, Tunis, Tunisia
Tel: +216-1-575-575
Fax: +216-1-237-076
E-mail: sa.benecib@planet.tn

STEINMAN, Harris, Allergy Clinic, Red Cross Children's Hospital, University of Cape Town, c/o P.O. Box 565, Milnerton, 7435, South Africa
Tel / Fax: +27-21-551-2993
E-mail: harris@zingsolutions.com

TRYPHONAS, Helen, Toxicology Research Division, Bureau of Chemical Safety, Food Directorate, Health Products and Food Branch, Health Canada, Sir Frederick G.Banting Research Center PL2202D1, Ross Avenue, Tunney's Pasture, Ottawa, Ontario, Canada
Tel: +1-613-957-0996
Fax: +1-613-941-6959
E-mail: Helen_Tryphonas@hc-sc.gc.ca

AUTHORS OF WORKING PAPERS

AALBERSE, Rob[4], Department of Immunopathology, CLB, Plesmanlaan 125, 1066 CX Amsterdam, The Netherlands
Tel: +31-20-512-3158
Fax: +31-20-512-3170
E-mail: aalberse@clb.nl

BECKER, Wolf-Meinhard[5]**,** Division of Allergology, Research Institute Borstel, Parkallee 35, D-23845 Borstel, Germany
Tel: +49-4537-188-337
Fax: +49-4537-188-328
E-mail: wbecker@fz-borstel.de

BINDSLEV-JENSEN, Carsten[6]**,** Associate professor, Odense University Hospital, Dept. of Dermatology, DK 5000 Odense, Denmark
Tel: +45 65411343, secr +45 65412717
Fax: +45 66123819
E-mail cbj@imbmed.sdu.dk

HELM, Ricki M.[7]**,** Associate Professor of Paediatrics, University of Arkansas for Medical Sciences, Arkansas Children's Hospital Research Institute, 1120 Marshall Street, Little Rock, Arkansas, USA,
Tel: +1-501-320-1060
Fax: +1-501-320-3173
E-mail: HelmRickiM@uams.edu

HILL, David J.[8]**,** Director, Department of Allergy, Royal Children's Hospital, 151 Flemington Road, NORTH MELBOURNE 3051, Australia
Tel: +61-3-9345-5701
Fax: +61-3-9326-6418
E-mail: allergy@cryptic.rch.unimelb.edu.au

PENNINKS, André H.[9]**,** Department of Experimental Immunology, TNO Nutrition and Food Research Institute, Utrechtweg 48, P.O. Box 360, 3700 AJ Zeist, The Netherlands
Tel: + 31 30 6944564
Fax: + 31 30 6960264
E-mail: Penninks@voeding.tno.nl

4 author of Topic 3
5 author of Topic 4
6 author of Topic 6
7 author of Topic 5
8 author of Topic 7
9 author of Topic 8

TAYLOR, Steve[10], Professor and Head, Department of Food Science and Technology, University of Nebraska, 143 Food Industry Complex, East Campus
PO Box 830919, Lincoln, NE 68583-0919, USA (Rapporteur)
Tel: +1 402 472 2833
Fax: +1 402 472 1693
E-mail: Staylor2@unl.edu

URISU, Atsuo[11]**,** Department of Pediatrics, Fujita Health University, The Second Teaching Hospital, 3-6-10, Otobashi, Nakagawa-ku, Nagoya, 454-8509 Japan,
Tel: +81-52-323-5670
Fax: +81-52-322-4734
E-mail: urisu@fujita-hu.ac.jp

WAL, Jean-Michel[12]**,** Directeur du Laboratoire d'Immuno-Allergie Alimentaire, Service de Pharmacologie et Immunologie (SPI), INRA-CEA SACLAY Bât 136,91191 Gif sur Yvette cedex, France
Tel: +33 1 69 08 92 24
Fax: +33 1 69 08 59 07
E-mail : wal@dsvidf.cea.fr

OBSERVERS FROM INTERNATIONAL ORGANIZATIONS

FERRAIOLO, Giovanni, Programme Officer, Biosafety Unit, International Center for Genetic Engineering and Biotechnology, Padriciano 99, 34012 Trieste, Italy,
Tel: +39-040-3757364
Fax: +39-040-226555
E-mail: ferraiol@icgeb.trieste.it

MAEKAWA, Tetsuya, Adminisrator, OECD, ENV/EHS
2, rue André Pascal, 75775 Paris Cedex 16, France
Tel: +33-1-45-24-76-19
Fax: +33-1-45-24-16-75
E-mail: Tetsuya.MAEKAWA@oecd.org

CHAIRMAN OF THE CODEX AD HOC TASK FORCE ON FOODS DERIVED FROM BIOTECHNOLOGY

YOSHIKURA, Hiroshi, Food Sanitation Division Environmental Health Bureau
Ministry of Health and Welfare
1-2-2 Kasumigaseki, Chiyoda-ku, Tokyo 100-8045, Japan
Tel: +81 3 3595 2252
Fax: +81 3 3595 2251
Email: codexj@mhw.go.jp

[10] author of Topic 1
[11] author of Topic 2
[12] author of Topic 9

CHAIRPERSON OF THE CODEX COMMITTEE ON FOOD LABELLING

MACKENZIE, Anne, Associate Vice-President, Science Evaluation Unit, Canadian Food Inspection Agency,
59 Camelot Drive, Nepean, Ontario K1A 0Y9, Canada
Tel: +1-613-225-2342, ext. 4188
Fax: +1-613-228-6638
E-mail: amackenzie@em.agr.ca

FAO/WHO SECRETARIAT

BOUTRIF, Ezzeddine, Senior Officer (Food Control and Consumer Protection), Food Quality and Standards Service, FAO
Viale delle Terme di Caracalla, 00100 Rome, Italy
Tel: +39 06 570 56156
Fax: +39 06 570 54593
E-mail: ezzeddine.boutrif@fao.org

TABATA, Makoto, Food Standards Officer, Joint FAO/WHO Food Standards Programme, Food and Nutrition Division, FAO
Viale delle Terme di Caracalla, 00100 Rome, Italy
Tel: +39 06 570 54796
Fax: +39 06 570 54593
E-mail: makoto.tabata@fao.org

LEE, Seoung-Yong, Associate Professional Officer, Joint FAO/WHO Food Standards Programme, Food and Nutrition Division, FAO
Viale delle Terme di Caracalla, 00100 Rome, Italy
Tel: +39 06 570 56243
Fax: +39 06 570 54593
E-mail: SeoungYong.Lee@fao.org

SAHARA, **Yasuyuki,** Scientist, Programme of Food Safety, WHO, 20 Avenue Appia, 1211 Geneva 27, Switzerland
Tel: +41 22 791 4324
Fax: +41 22 791 4807
E-mail:saharay@who.int

EIJKEMANS, Gerry, Medical Officer, Department of Protection of Human Environment, WHO, 20 Avenue Appia, 1211 Geneva 27, Switzerland
Tel: +41 22 791 3758
Fax: +41.22.791 4123
E-mail: eijkemansg@who.int

JERMINI, Marco, Acting Director and Food Safety Regional Adviser
WHO Regional Office for Europe - European Centre for Environment and Health
Via Francesco Crispi, 10 I-00187 Rome Italy
Tel: +39-06-487-7525
Fax: +39-06-487-7599
E-mail: maj@who.it

Annex 2

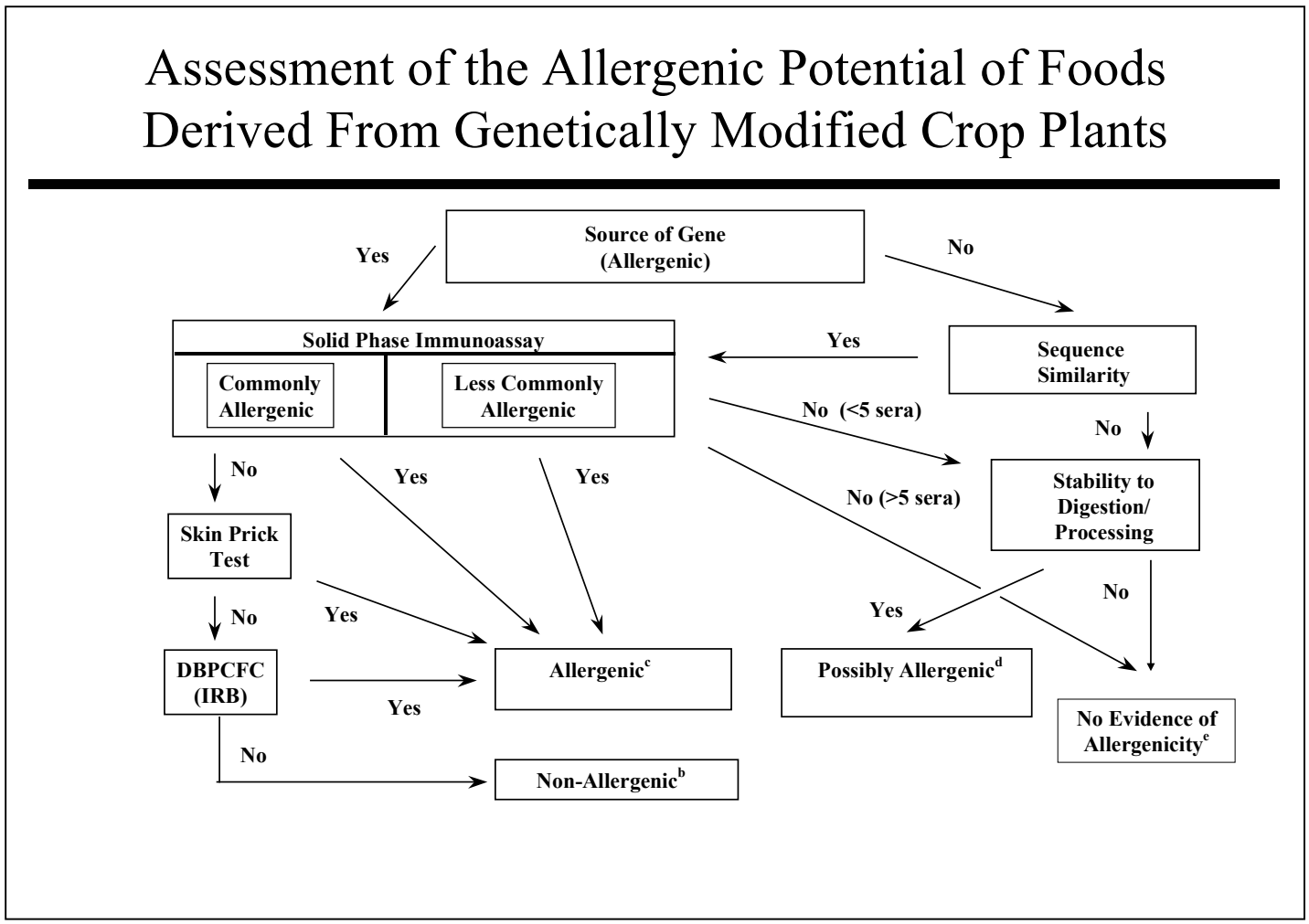
List of Documents[13]

Biotech 01/01	Provisional Agenda and Timetable
Biotech 01/02	Questions about the Assessment of Allergenicity of Foods Derived from Modern Biotechnology
Biotech 01/03	Topic 1: Overview of the Current Approach to Determine the Allergenicity of Genetically Modified Foods (Decision Tree Approach)
Biotech 01/04	Topic 2: Commonly Known Allergenic Sources (IgE-Mediated and Non IgE-Mediated Food Allergens as well as Environmental Allergens)
Biotech 01/05	Topic 3: Allergen Databases/Class of Proteins/Allergen Function
Biotech 01/06	Topic 4: Sequence Homology and Allergen Structure
Biotech 01/07	Topic 5: Stability of Known Allergens (Digestion and Heat Stability
Biotech 01/08	Topic 6: Solid phase Immunoassay, Immunoreactivity and Other Criteria
Biotech 01/09	Topic 7: Prevalence of Allergen in Food and Threshold for Sensitization
Biotech 01/10	Topic 8: Animal Model for Allergenicity Assessment
Biotech 01/11	Topic 9: Post-market Surveillance of Allergenicity

[13] Working Documents are posted on the following FAO and WHO websites:
FAO : http://www.fao.org/WAICENT/FAOINFO/ECONOMIC/ESN/biotech.htm
WHO: http://www.who.int/fsf

Annex 3

FAO/WHO 2000 Decision Tree



Footnotes to Figure

(a) The figure was adapted from decision-tree approach developed by International Food Biotechnology Council and Allergy and Immunology Institute of the International Life Sciences Institute (Metcalfe *et al*., 1996).

(b) The combination of tests involving allergic human subjects or blood serum from such subjects would provide a high level of confidence that no major allergens were transferred. The only remaining uncertainty would be the likelihood of a minor allergen affecting a small percentage of the population allergenic to the source material.

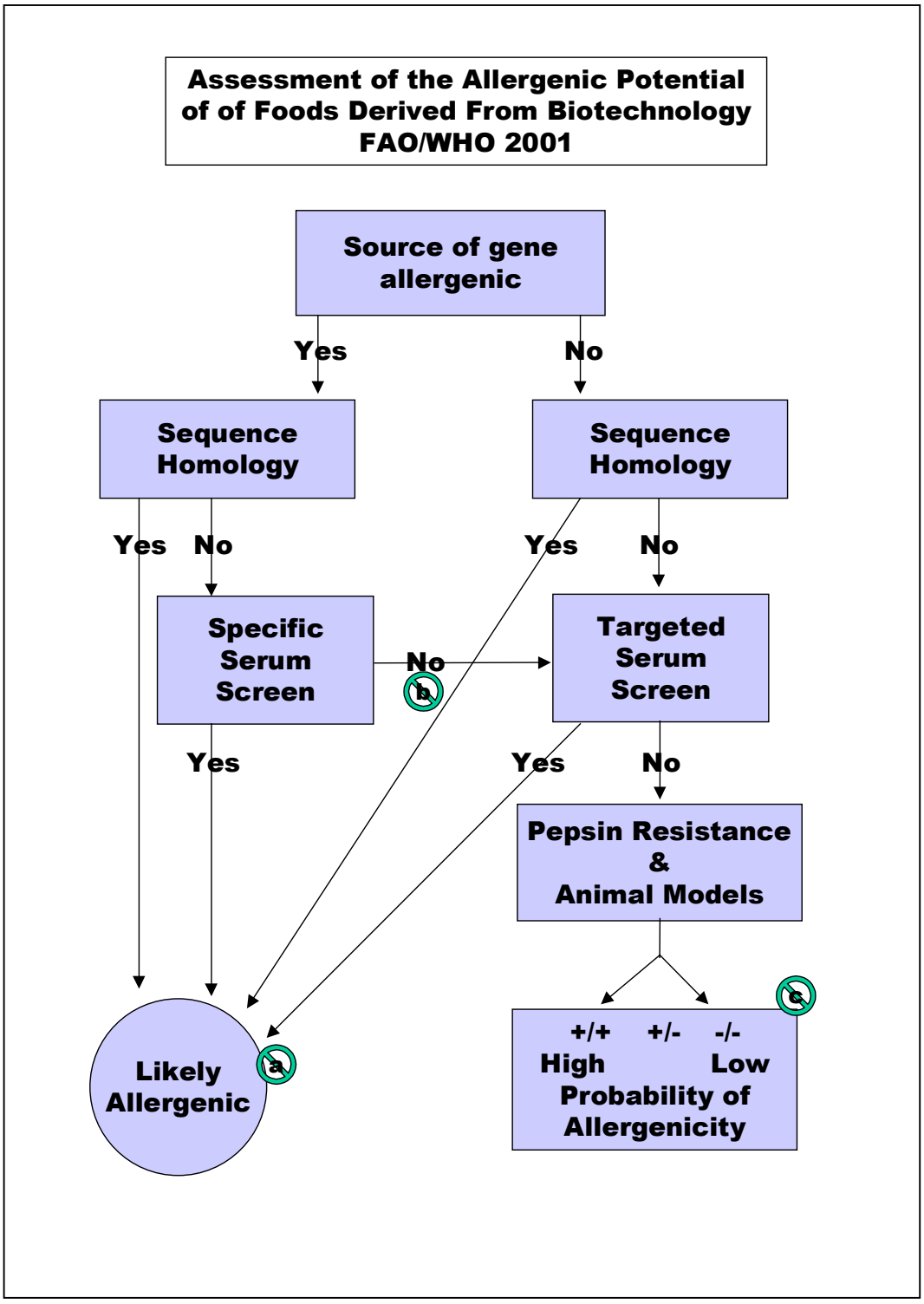
(c) Any positive results obtained in tests involving allergenic human subjects or blood serum from such subjects would provide a high level of confidence that the novel protein was a potential allergen. Foods containing such novel proteins would need to be labelled to protect allergic consumers.

(d) A novel protein with either no sequence similarity to known allergens or derived from a less commonly allergenic source with no evidence of binding to IgE from the blood serum of a few allergic individuals (<5), but that is stable to digestion and processing should be considered a possible allergen. Further evaluation would be necessary to address this uncertainty. The nature of the tests would be determined on a case-by-case basis.

(e) A novel protein with no sequence similarity to known allergens and that was not stable to digestion and processing would have no evidence of allergenicity. Similarly, a novel protein expressed by a gene obtained from a less commonly allergenic source and demonstrated to have no binding with IgE from the blood serum of a small number of allergic individuals (>5 but <14) provides no evidence of allergenicity. Stability testing may be included in these cases. However, the level of confidence based on only two decision criteria is modest. The Consultation suggested that other criteria should also be considered such as the level of expression of the novel protein.

Annex 4

FAO/WHO 2001 Decision Tree



Footnotes

[a] Any positive results obtained from sequence homology comparisons to the sequences of known allergens in existing allergen databases or from serum screening protocols, both conducted in accordance with the guidelines established in Sections 6.1, 6.2 and 6.3, indicate that the expressed protein is likely allergenic.

[b] The degree of confidence in negative results obtained in the specific serum screen is enhanced by the examination of larger numbers of individual sera as explained in Section 5.3. Conducting the specific serum screen with small numbers of individual sera when larger numbers of such sera are readily available should be discouraged.

[c] When positive results are obtained in both the pepsin resistance and animal model protocols, the expressed protein has a high probability to become an allergen. When negative results are obtained in both protocols, the expressed protein is unlikely to become an allergen. When different results are obtained in the pepsin resistance and animal model protocols, the probability of allergenicity is intermediate, although rational explanations may be possible in some situations.