Food and Agriculture Organization
of the United Nations

World Health Organization

Safety aspects of genetically modified foods of plant origin

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

World Health Organization, Headquarters
Geneva, Switzerland
29 May – 2 June 2000

CONTENTS

1. Introduction

A Joint FAO/WHO Expert Consultation on Foods Derived from Biotechnology was held at the Headquarters of the World Health Organization (WHO) in Geneva from 29 May to 2 June 2000. A total of 27 experts, including authors of discussion papers, participated in the Consultation. The complete list of participants is given in Annex 1.

Ms Poonam Khetrapal Singh, Executive Director, Cluster of Sustainable Development and Healthy Environments, opened the Consultation on behalf of Directors-General of WHO and the Food and Agriculture Organization of the United Nations (FAO). In her statement, Ms Singh indicated that biotechnology would provide powerful tools for the sustainable development of agriculture and food production. When appropriately integrated with other technologies for the food production, biotechnology can be of significant assistance in meeting the needs of an expanding and increasingly urbanized population in the new millennium. Ms Singh drew attention to the concern expressed by the public at large over the safety and nutritional aspects of foods derived from biotechnology. She stressed the need for the constant review of methodologies for risk assessment and expected the Consultation to provide Member States of FAO and WHO with useful guidance in this respect.

The Consultation elected Dr Harry Kuiper as Chairperson and Dr Marília Regini Nutti as Vice-Chairperson. Dr James Maryanski was elected as Rapporteur and Dr Jennifer Thomson as Vice-Rapporteur. The Consultation decided to nominate Co-Rapporteurs for each of the sections of the programme to assist in the drafting of the report of the Consultation. Dr Ronald Walker was nominated Co-Rapporteur for Section A (Approaches to the Nutritional and Food Safety Evaluations), Dr Keith Downey for Section B (Nutrition-Related Issues) and Dr Ian Munro for Section C (Specific Food Safety Issues)[1].

2. Background

Previous expert Consultations convened by FAO/WHO and OECD have 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 (OECD, 1993; FAO, 1996). This concept embodies a science-based approach in which a genetically modified food is compared to its existing, appropriate counterpart. The approach is not intended to establish absolute safety, which is an unattainable goal for any food. Rather, the goal of this approach is to ensure that the food, and any substances that have been introduced into the food as a result of genetic modification, is as safe as its traditional counterpart.

Several countries have used the concept of *substantial equivalence* as an important component of the safety evaluations of foods and food ingredients derived from genetically modified plants. They have found this approach to be scientifically sound and practical. Nevertheless, there has not been a universal consensus on the application of this concept. This has resulted in criticism that the approach does not provide a sufficient basis for safety and calls for national governments and international bodies to consider alternative approaches.

FAO and WHO convened this Consultation to evaluate experience gained since the 1996 Joint FAO/WHO Consultation (FAO, 1996) and to assess whether any new scientific information would suggest a need for modifying current approaches for assessing the safety of foods and food ingredients derived from genetically modified plants. This Consultation also provided an

[1] The list of working documents of the Consultation is reproduced in Annex 2 of this report.

opportunity, in the light of recent scientific reports, to review the scientific basis, application, and limitations of the concept of *substantial equivalence*.

3. Scope

The Consultation was convened to address food safety and nutritional questions regarding foods and food ingredients derived from plants that have been genetically modified using recombinant DNA techniques. Nevertheless, the concepts and principles described in this report are equally applicable to all foods and food ingredients derived from plants modified by other techniques.

For the purpose of this report, the term "genetically modified food/plant" is used to describe foods or food ingredients that are, or are derived from, plants that have been modified (engineered) through the use of recombinant DNA techniques.

Specifically, the Consultation was requested:

- to provide FAO, WHO and their Member States with scientific support in relation to the safety and nutritional features of foods derived from biotechnology on the basis of available scientific data, taking into consideration work done by national authorities, FAO, WHO and other international organisations and other relevant international fora;
- to review existing strategies for the safety and nutritional assessment of foods derived from biotechnology, taking into account ever increasing public concerns and experiences accumulated in testing such foods;
- to make recommendations on further research needs and priorities for evaluation of safety and nutritional aspects of foods derived from biotechnology.

The first session of the Codex *ad hoc* Intergovernmental Task Force on Foods Derived from Biotechnology, which had been established by the twenty-third session of the Codex Alimentarius Commission in June/July 1999, was held in Chiba, Japan in March 2000. It welcomed the initiative of FAO and WHO to convene the Consultation. It identified the following questions on which the Consultation was invited to formulate scientific opinions:[2]

- What overarching scientific principles should be applied to the safety and nutritional assessment?
- What is the role, and what are the limitations, of *substantial equivalence* in the safety and nutritional assessment? Are there alternative strategies to *substantial equivalence* that should be used for the safety and nutritional assessment?
- What scientific approach can be used to monitor and assess possible long-term health effects or unintended/unexpected adverse effects?
- What scientific approach can be used to assess the potential allergenicity?
- What scientific approach can be used to assess the possible risks arising from the use of antibiotic resistance marker genes in plants and microorganisms?

The Consultation did not consider environmental safety issues related to the release of genetically modified food/plants into the environment as these were outside its defined scope. Similarly, it did not consider non-science aspects such as socio-economics, risk management and public acceptance.

[2] The reply to the questions by the Consultation is reproduced in Annex 3 of this report.

4. Approaches to the Nutritional and Food Safety Evaluation of Genetically Modified Foods

4.1 Introduction

For many years the practical difficulties of obtaining meaningful information from conventional toxicology studies on the safety of whole foods have been well recognized (OECD, 1996). The limitations of conventional toxicological studies became particularly apparent when animal feeding studies were used to assess the safety of irradiated foods.

Animal studies are a major element in the safety assessment of many compounds such as pesticides, pharmaceuticals, industrial chemicals and food additives. In most cases however, the test substance is well characterized, of known purity, of no particular nutritional value and human exposure 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 determine levels of exposure at which adverse effects are not observed, and so set safe upper limits by the application of appropriate safety factors.

By contrast, foods are complex mixtures of compounds characterised by 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, to try 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. 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.

In practice, very few foods consumed today have been subject to any toxicological studies, yet they are generally accepted as being safe to eat. In developing a methodology for the safety assessment of new foods, it was essential to establish a benchmark definition of safe food. This was taken up by OECD in 1991 who said that food is considered safe if there is reasonable certainty that no harm will result from its consumption under anticipated conditions of use.

The difficulties of applying traditional toxicological testing and risk assessment procedures to whole foods, meant that an alternative approach was required for the safety assessment of genetically modified foods. This led to development of the concept of *substantial equivalence*.

This approach acknowledges that the goal of the assessment is not establishing absolute safety but to consider whether the genetically modified food is as safe as its traditional counterpart, where such a counterpart exists.

The Consultation agreed that the practical difficulties already identified in relation to the application of conventional toxicology studies to whole food preclude their use as a routine safety assessment technique for genetically modified foods. The Consultation also recognised that the use of toxicology studies could not be justified from an animal welfare perspective where it was unlikely to result in meaningful information. In addition, the Consultation noted that the concept of *substantial equivalence* was a good example of an approach to reduce the use of animals in toxicology studies by refining safety assessment techniques and replacing animal models with alternatives.

The concept of *substantial equivalence* was developed as a practical approach to the safety assessment of genetically modified foods. The Consultation agreed that *substantial equivalence* should be seen as a key step in the safety assessment process. The application of the concept is not a safety assessment in itself; it does not characterize the hazard, rather it is used to structure the safety assessment of a genetically modified food relative to its conventional counterpart. As a starting point, the genetically modified organism (plant, micro-organism or animal), and/or foods derived from it, is compared with its closest traditional counterpart in order to identify any intended and unintended differences which then become the focus of the safety assessment. Data for comparison should be obtained using validated methods and analyzed using appropriate statistical techniques. The comparative approach should take into account agronomic, genetic and chemical aspects and only when all of these have been considered can an objective assessment of safety be made. The type and extent of further studies depend on the nature of the differences and whether or not they are well characterised. Studies should be carried out in accordance with good laboratory practise.

4.2 Safety Assessment

4.2.1 Basic Principles

Several international organisations have already addressed the issues associated with the safety assessment of novel foods and, in the present context, genetically modified plants and micro-organisms (WHO, 1991; OECD, 1993; WHO, 1995; FAO, 1996; ILSI, 1996; Commission of the European Communities, 1997). It is generally agreed that such an assessment requires an integrated and stepwise, case-by-case approach and some authorities have developed decision trees to assist in determining the extent of testing required in specific cases (SCF, 1997; UK ACNFP, 1995). This approach is useful in determining appropriate safety assessment strategies.

The safety assessment of a genetically modified food is directed by the results of a comparison between the genetically modified food and its conventional counterpart. It follows a stepwise process aided by a series of structured questions. Factors taken into account in the safety assessment include:

- identity;
- source;
- composition;
- effects of processing/cooking;
- transformation process;
- the recombinant DNA (e.g. stability of insertion, potential for gene transfer);
- protein expression product of the novel DNA;
    - effects on function;
    - potential toxicity;
    - potential allergenicity;
- possible secondary effects from gene expression or the disruption of the host DNA or metabolic pathways, including composition of critical macro-, micro-nutrients, anti-nutrients, endogenous toxicants, allergens, and physiologically active substances; and,

- potential intake and dietary impact of the introduction of the genetically modified food.

The above factors are particularly pertinent to the assessment of foods derived from genetically modified plants. When assessing the safety of foods derived from genetically modified animals and micro-organisms, other factors may need to be taken into account on a case-by-case basis.

4.2.2. Need for Animal Studies

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.

4.3 Unintended Effects

In achieving the objective of conferring a specific target trait (intended effect) to the host organism by the insertion of defined DNA sequences, additional traits could, theoretically, be acquired or existing traits lost (unintended effects). The assessment of genetically modified foods involves methods to detect such unintended effects and procedures to evaluate their biological relevance and their impact on food safety.

Unintended effects may be due to factors such as random insertion events which might result in disruption of existing genes, modifications of protein expression or formation of new metabolites. The expression of enzymes at high levels may give rise to secondary biochemical effects, e.g. an altered metabolic flux resulting in changed metabolite patterns.

The potential occurrence of unintended effects is not specific to the use of recombinant DNA techniques. Rather, it is an inherent and general phenomenon that can occur in conventional breeding. One of the approaches adopted to cope with this problem is to select/ discard plants with unusual and undesired phenotypic and agronomic parameters at an early stage of the plant variety development. The practice of consecutive back-crossing is also a common procedure used to eliminate unintended effects.

Unintended effects due to genetic modification may be subdivided into two groups: those which are "predictable" based on metabolic connections to the intended effect or knowledge of the site of insertion and those which are “unexpected”. Due to the increased precision of genetic modification compared to conventional breeding, it may become easier to predict pathways likely to be influenced by unintended effects.

The comparator used to detect unintended effects should ideally be the near isogenic parental line grown under identical conditions. In practice, this may not be feasible at all times, in which case a line as close as possible should be chosen. The resulting natural variation should be taken into account in assessing the statistical significance of the unintended effect.

Where statistically significant unintended differences are observed, their biological significance should be assessed. This may be assisted by knowledge of the mechanisms leading to the changes. In order to assess the biological and safety relevance of an unintended effect, data on the genetically modified plant should be compared to data on other conventional varieties and literature data. If the differences exceed natural variations in traditional food crops, further assessment is required.

Present approaches to assess possible unintended effects are based, in part, on the analysis of specific components (targeted approach). In order to increase the probability of detecting unintended effects, profiling techniques are considered as useful alternatives (non-targeted approach). Profiling techniques are used at different level e.g. genomics, proteomics and metabolomics, and may contribute to the detection of differences in a more extensive way than targeted chemical analysis. However, they are not yet fully developed and validated and have certain limitations.

In the future, genetic modifications of plants are likely to be more complex perhaps involving multiple between-species transfers and this may lead to an increased chance of

unintended effects. Where differences are observed using profiling techniques, the possible implications of the differences with respect to health need to be considered.

4.4 Evaluation of the Concept of *Substantial Equivalence*

The Consultation acknowledged that the concept of *substantial equivalence* had attracted criticism. This criticism relates, in part, to the mistaken perception that the determination of *substantial equivalence* was the end point of a safety assessment rather than the starting point. Further disagreement may have arisen from reference to three outcomes of *substantial equivalence* discussed previously (i.e. *substantially equivalent, substantially equivalent* apart from defined differences, and not *substantially equivalent*) (FAO, 1996).

Having considered the way in which the concept of *substantial equivalence* is currently used, and the possible use of alternative strategies, the Consultation concluded that application of the *substantial equivalence* concept contributes to a robust safety assessment framework. The Consultation was satisfied with the approach used to assess the safety of the genetically modified foods that have been approved for commercial use.

It was agreed that communication of the principles involved in safety assessment could be improved. The Consultation concluded that the key message to be conveyed is that *substantial equivale*nce is a concept used to identify similarities and differences between the genetically modified food and a comparator with a history of safe food use which subsequently guides the safety assessment process.

The Consultation reiterated that a consideration of compositional changes was not the sole basis for determining safety. Safety can only be determined when the results of all aspects under comparison are integrated.

It was recognised that whole foods do not lend themselves to the standard safety evaluation principles (WHO 1987) used for food additives and other chemicals and that a quantitative assessment of risk of individual whole foods from whatever source cannot be achieved. The Consultation agreed that assessing safety relative to existing foods offered the best means of assessing the safety of genetically modified foods.

The Consultation considered the issue of long term effects from the consumption of genetically modified foods and noted that very little is known about the potential long term effects of any foods. In many cases, this is further confounded by wide genetic variability in the population, such that some individuals may have a greater predisposition to food-related effects.

In this context, the Consultation acknowledged that for genetically modified foods, the pre-marketing safety assessment already gives assurance that the food is as safe as its conventional counterpart. Accordingly it was considered that the possibility of long term effects being specifically attributable to genetically modified foods would be highly unlikely. Furthermore, it was recognised that observational epidemiological studies would be unlikely to identify any such effects against a background of undesirable effects of conventional foods. Experimental studies, such as randomised controlled trials (RCTs), if properly designed and conducted, could be used to investigate the medium/long term effects of any foods, including genetically modified foods. Such studies could provide additional evidence for human safety, but would be difficult to conduct. In this respect, it is also important to recognise the wide variation in diets and dietary components from day to day and year to year.

The Consultation was of the view that there were presently no alternative strategies that would provide a better assurance of safety for genetically modified foods than the appropriate use

of the concept of *substantial equivalence*. Nevertheless, it was agreed that some aspects of the steps in the safety assessment process could be refined to keep abreast of developments in genetic modification technology. New methodologies, such as profiling techniques, offer the means of providing a more detailed analytical comparison. However, it was recognised that much more developmental work was necessary before such methods could be validated.

5. Nutrition-Related Issues

5.1 Introduction

The variety of foods consumed by humans has changed greatly over the centuries, altering the balance of nutrients in the diet. Plant breeding by traditional methods, mutations and recombinant DNA technique can be used to alter nutritional quality and functional traits. Thirty years ago, traditional breeding was utilized to identify and select rapeseed plants free of the nutritionally undesirable fatty acid, erucic acid. The resulting plant, canola, produced an oil that has a more desirable fatty acid profile. Canola oil now makes up a significant proportion of the daily lipid intake of the consumers in most of the developed nations of the world. More recently, mutational breeding has been used to genetically modify flax plants to produce edible oils instead of the traditional high linolenic industrial oil. The introduction of new foods and the growing interest in functional foods also have the potential to modify the food supply. The availability of recombinant DNA techniques provides the opportunity to develop foods that help optimize health status. The major difference with recombinant DNA techniques is the ability to introduce different nutrient profiles with greater speed and precision.

Genetic modification of plants now under cultivation have been directed towards agronomic enhancement. Nutritional changes may have a more profound impact on the health of the population. At present, there are no foods derived from genetically modified plants modified to enhance nutrition in the commercial market. However, there are several plants with altered nutrient composition being developed using recombinant DNA technology. These have been designed to modify nutrient composition and levels or change the functionality of a product. An example of the latter is potato tubers containing increased amounts of starch that is distributed more uniformly, resulting in more efficient processing, lower fat absorption and improved texture. It is anticipated that other genetically modified plants will be developed with nutritional characteristics targeting major health problems.

There are a number of examples of genetically modified plants from which foods are produced with the objective of improving health and enhancing food functionality. These include two examples of genetically modified rice varieties, one in which beta-carotene was produced and another in which an undesirable component for sake brewing (glutelin) was decreased. Other examples were oil seeds in which the fatty acid profile was changed either by traditional mutational techniques or recombinant DNA technique. Recently, canola and soy oils with combined low levels of saturated fatty acids and increased oleic acid, have been produced with the objective of lowering total and low density lipoprotein (LDL) cholesterol levels, one of the risk factors for cardiovascular disease, while at the same time enhancing the functionality of the oil. The Consultation heard details of the recently reported "golden rice" which was specifically designed to target Vitamin A deficiency, a cause of blindness among people living in developing countries (Ye *et al*., 2000).*)* These examples highlight the potential of foods with modified nutritional profiles to reduce the incidence of nutrition-related conditions or diseases.

5.2 Unexpected Outcomes: Targeting a Single Nutrient can Result in Other Alterations

Traditional plant breeding techniques of intra and inter-species crossing and mutation are designed to create genetic variation upon which selection of the most desired genotype is the expected outcome. All plant breeding procedures can produce unexpected effects. Low glutelin genetically modified rice, created using an anti-sense technique, signifies improvements in rice storage proteins for commercial sake brewing. The decrease in glutelin levels was however associated with an unintended increase in levels of prolamins. This illustrated that a targeted change in the level of a specific protein can result in other proteins being affected. The change in prolamin levels did not affect the industrial application but could affect nutritional quality and allergenic potential if the rice were used as a food. Other examples were given regarding soybean and rice showing that genetically modified soy with increased lysine showed an unexpected decrease in oil content, and the genetically modified "golden rice" designed to express beta-carotene unexpectedly accumulated xanthophylls. The use of tissue-specific promoters was suggested as a means to limit the number and extent of unexpected effects.

In the case of the low glutelin rice, the change in prolamins would not be detected by standard nutritional analyses such as total protein and amino acid profiles. This was only observed following sodium dodecyl sulphate (SDS) gel electrophoresis. The unexpected finding of xanthophylls in the beta-carotene-enhanced genetically modified rice would not have been apparent from standard nutritional analyses. This difference was observed following high pressure liquid chromatography (HPLC) analyses for carotenoids. Thus, it is important that appropriate analyses of nutrients should be carried out to identify unexpected changes.

The ability to change nutrient levels in crop plants through plant breeding, including the use of recombinant DNA techniques, has the potential to result in broad changes in at least two ways: (1) the intended modification in plant constituents could change the overall nutrient profile of the plant product and this change could affect nutritional status of the individual, (2) in addition, unexpected alterations in nutrients could also affect nutrient profiles of the product and nutritional status of people. Although the genetically modified plant components may be assessed as safe individually, the impact of the change on the overall nutrient profile must be determined. Because changes in individual nutrients could affect a number of plant processes and nutritional outcomes, it is recommended that integration of nutritional and toxicological expertise needed for the evaluation of genetically modified foods be encouraged and facilitated. Consideration should be given to assessing the potential health impact resulting from changes in nutrient profile arising from all types of plant breeding.

Examples of the use of mutational breeding to alter the nutritional characteristics of plants include the modification of flaxseed oil from a high linolenic industrial oil to a high linolenic oil similar to corn oil in its fatty acid composition, and genetically modified soy and canola plants being developed that produce a high oleic acid (80-90%) oil that also displays very low levels of saturated and polyunsaturated fatty acids.

It will be important to determine if the overall nutrient profile of a genetically modified food has been changed and if dietary intake patterns are altered by the introduction of foods from genetically modified plants. The introduction of a significant nutritional change in a food may require post-market assessment to determine whether the overall diet has been altered and to what degree, before an assessment of the impact on nutritional status can be made.

It is important to ascertain to what extent the modified nutrient is bioavailable and remains stable with time, processing, and storage. For example, the question was raised as to

what extent carotenoids in the genetically modified rice remained stable under storage conditions encountered in the developing countries.

5.3 Methodology for Nutritional and Safety Evaluation

Where additional assurance of safety is sought, analytical methods traditionally applied in the evaluation of food constituents such as total protein, fat, ash, fibre and micronutrients may need to be augmented with additional analyses to identify unexpected effects and altered nutrient profiles and bioavailability which may impact on dietary intake and health.

Because of 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 studies in animals to determine outcomes that result from changes in nutrient profiles and nutrient bioavailability. Nutritional modifications which are within the normal range of nutrient variation might require a less extensive evaluation than those outside normal ranges.

Genetically modified foods have the potential to improve the nutritional status of individuals and provide products with enhanced functionality for populations in developed and developing countries. The major issues relate to possible nutritional imbalances and the introduction of unexpected alterations in nutrients and other compounds. The change in nutrient levels in a particular crop plant may impact overall dietary intake. In such cases, it would be important to monitor changes in nutrient levels and bioavailability in such foods and evaluate their potential effect on nutritional and health status of consumers. However, an assessment of the impact on nutritional status of consumers is important for all significant dietary changes and not specific to the introduction of genetically modified foods.

6. Specific Food Safety Issues

6.1 Introduction

This section deals with specific issues that are frequently raised with regard to the safety of genetically modified foods. These issues include the potential for gene transfer from genetically modified plants to gut microflora and mammalian cells, the safety of antibiotic resistance genes as markers for the selection of genetically modified plants, and the assessment of the potential allergenicity of genetically modified foods that may be caused by the presence in these foods of novel gene products. The discussion which follows provides an evaluation of existing knowledge about these topics and elaborates scientific approaches that may be used to assess possible health risks.

6.2 Gene Transfer from Genetically Modified Plants: Mechanisms and Consequences for Food Safety

As background to the discussion that follows, it should be noted that all foods contain DNA, which is ingested in significant quantities. In humans, dietary intakes of RNA and DNA vary widely but are typically in the range from 0.1 to 1.0 g per day (Doerfler and Schubbert, 1997). Any concerns over the presence of novel DNA in a genetically modified food consumed in the human diet must take into consideration that this DNA would represent less than 1/250,000 of the total amount of DNA consumed. In view of this and the digestibility of dietary DNA, the probability of transfer of genes from genetically modified plants to mammalian cells is extremely low. It is nevertheless necessary to examine this possibility and the consequences of such transfer if it were to occur.

The transfer of plant DNA into microbial or mammalian cells under normal circumstances of dietary exposure would require all of the following events to occur:

- the relevant gene(s) in the plant DNA would have to be released, probably as linear fragments;
- the gene(s) would have to survive nucleases in the plant and in the gastrointestinal tract;
- the gene(s) would have to compete for uptake with dietary DNA;
- the recipient bacteria or mammalian cells would have to be competent for transformation and the gene(s) would have to survive their restriction enzymes; and
- the gene(s) would have to be inserted into the host DNA by rare repair or recombination events.

There have been numerous experiments aimed at evaluating the possibility of transfer of plant DNA to microbes and mammalian cells. To date, there are no reports that marker genes in plant DNA transfer to these cells. Results of model experiments in which mice were orally administered high doses of bacterially derived DNA indicated apparent incorporation of the test DNA fragments into bacterial and mouse cells (Schubbert *et al.*, 1998). The report contrasts with other reports where no transfer or only a low frequency of transfer was observed. The significance of the observations of Schubbert *et al.* have been seriously questioned (Beever and Kemp, 2000). It was concluded that the data do not demonstrate that plant DNA can be

transferred to and stably maintained in mammalian cells. There is additionally no evidence that intact genes from plants can be transferred to and be expressed in mammalian cells.

It should be noted that the vast majority of known bacteria are not naturally transformable and there is as yet no evidence of transfer to and expression of plant genes in bacteria under natural conditions. Transfer has been observed under laboratory conditions, but only if homologous recombination is possible (Nielsen *et al.,* 1998). It should be noted that inserted gene sequences in genetically modified plants show in many cases homology with prokaryotic genes. The Consultation is aware of the study being undertaken whereby chickens and sheep are being fed genetically modified maize, and bacteria in the normal flora of the gastrointestinal tract are being tested for DNA uptake.

Should horizontal gene transfer from a genetically modified plant to bacteria occur, the gene (e.g. an antibiotic resistance gene) may alter the fitness of the recipient cell. A reduction in fitness may not provide sufficient selective pressure to eliminate the gene or gene fragment from the gene pool. The presence of this DNA in the cell population could then serve as a genetic reserve for the evolution of the recipient species.

The available knowledge on bacteria is derived from bacteria that can be cultured and readily analyzed. Bacteria that are susceptible neither to culture nor identification represent a significant proportion of existing microflora. Therefore, without available knowledge of these bacteria, it is not possible to assess the possibility, probability or consequences of their acquisition of genes or gene fragments.

The consequences of uptake of plant DNA by mammalian cells are different from those of uptake by bacteria because existing data indicate that such DNA is not transmitted via the germline. The extent to which cells containing exogenous DNA are phagocytosed is not yet clear. Neither is it clear that the incorporated DNA is stably maintained and replicated in somatic cells. Mammalian cells would be similarly affected by uptake of exogenously derived DNA regardless of its source.

The most important consideration with respect to horizontal gene transfer is the consequence of a gene being transferred from a genetically modified plant and expressed in recipient cells. Therefore data on the possible extent of such transfer will be needed as part of the safety assessment when the nature of the transferred gene(s) is such that, if transfer were to occur, it would give rise to health concerns.

The Consultation noted that the antibiotic resistance markers currently used in genetically modified plants have been previously reviewed for safety (WHO, 1993). There is no evidence that the markers currently in use pose a health risk to humans or domestic animals. Nevertheless, with the variable gene transfer frequencies noted in current literature, the transfer and expression of a functional antibiotic resistance gene to recipient cells, while remote, cannot be ignored. If the recipient cell is subjected to selection from therapeutic use of the antibiotic, proliferation of a drug resistant cell population could compromise the effectiveness of the drug. This directs attention to the more important considerations: whether there are already prevailing high levels of culturable bacteria resistant to that antibiotic, whether that antibiotic is, or could be, clinically important, and whether there are alternative effective therapies.

For certain antibiotic resistance genes currently in use in genetically modified plants, the available data suggest that the consequences of horizontal gene transfer will be unlikely to pose a significant threat to the current therapeutic use of the respective drugs. With other genes that confer resistance to drugs that are important in specific medical use, or to drugs that have limited alternative therapies, the possibility of transfer and expression of these genes is a risk that

warrants their avoidance in the genomes of widely disseminated genetically modified organisms and foods and food ingredients.

A number of methods are available to genetically modify plants without incorporation of an antibiotic resistance gene in the commercial product. These methods include removing the gene after successful gene transfer, or using alternative marker genes for genetic transformation. If alternative marker genes are used, they also would need to be evaluated for safety. In addition, it is recognized that further technical development of these or additional methods may be necessary for practical transformation of certain plant species. In future developments, the Consultation encourages the use of alternative transformation technologies, if available and demonstrated to be safe, that do not result in antibiotic resistance genes in genetically modified foods. If further development of alternative technologies is required, additional research should be strongly encouraged.

6.3 Allergenicity

6.3.1 Introduction

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 allergies are mediated by allergen-specific immunoglobulin E (IgE) antibodies[3]. 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 10-25% of the population in developed countries (Mekori, 1996), although food allergies represent a small fraction of all allergic diseases. IgE-mediated food allergies affect less than 2.5% 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 may be as high as 5-8% (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, celiac disease, also known as gluten-sensitive enteropathy, affects 1 in every 300 to 3000 individuals in the population depending upon the specific geographic region.

The Codex Alimentarius Commission has adopted a list of the most common allergenic foods associated with IgE-mediated reactions on a world-wide basis that includes peanuts, soybeans, milk, eggs, fish, crustacea, wheat, and tree nuts. 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, 1996). Allergic reactions to fresh fruits and vegetables, the so-called oral allergy syndrome, are also rather common (Parker, 1990), but these foods are not included on the Codex Alimentarius Commission list because the symptoms are typically mild and confined to the

[3] IgE, or immunoglobulin E, is a protein antibody that recognizes an allergen. It 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 allergen, this triggers the release of chemical mediators that provoke the symptoms associated with allergic reactions.

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 etiology of gluten-sensitive enteropathy.

The symptoms of IgE-mediated food allergies can range from mild to severe and life-threatening. Individuals display different thresholds for the offending food, but the most sensitive food-allergic individuals will experience reactions from exposure to trace quantities of the offending food. Life-threatening reactions usually involve exposure to larger doses of the offending food.

Gluten-sensitive enteropathy is a mal-absorbtion syndrome characterized by body wasting, anaemia, diarrhoea, and bone pain along with other symptoms. The threshold dose needed to provoke the symptoms of gluten-sensitive enteropathy are unknown, but also thought to be quite low.

Both IgE-mediated food allergies and gluten-sensitive enteropathy are treated with specific avoidance diets. Since in both cases, the threshold dose is quite low, great care must be taken in the construction of safe and effective avoidance diets.

Almost all food allergens are proteins, although the possibility exists that other food components may act as haptens[4]. 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 ten of 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 crop improvement practices have had little, if any, effect on the allergenic potential of our major foods. In contrast, altered dietary preferences 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. 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.

6.3.2 Evaluation of the Potential Allergenicity of Novel Proteins in Genetically Modified Foods

In 1996, the International Food Biotechnology Council and the Allergy and Immunology Institute of the International Life Sciences Institute developed a decision-tree approach (Metcalfe *et al*., 1996). This allergy assessment strategy has been widely adopted by the agricultural biotechnology industry. This strategy 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 source of the transferred genetic material, and the physicochemical properties of the newly

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

introduced protein (Metcalfe *et al*., 1996; Taylor, 1997). This study has been adapted by the Consultation for the assessment of the allergenicity of genetically-modified foods [Figure 1].

Since genetically modified foods usually contain novel proteins, their safety should include an assessment of the allergenicity of such novel proteins. The current decision-tree approach requires the examination of a number of parameters which are common to many food allergens. These characteristics facilitate the identification of potentially allergenic gene products, although no single criterion is sufficient to confirm allergenicity or the lack thereof. The relevant criteria used in the current decision tree include:

- Source of the transferred genetic material: Particular caution must be exercised if the source of this material contains known allergens.
- Sequence homology: The amino acid sequence of many allergens is readily available.
- Immunoreactivity of the newly introduced protein: If the novel protein is derived from a known allergenic source or if it has sequence homology with a known allergen, then the reactivity of this novel protein with IgE from the blood serum of appropriate allergic individuals is determined.
- Effect of pH and/or digestion: Most allergens are resistant to gastric acidity and to digestive proteases.
- Heat or processing stability: Labile allergens in foods that are eaten cooked or undergo other processing before consumption are of less concern.

The desirability of including other relevant criteria to improve the reliability of the allergy assessment decision-tree approach was discussed. When the genetically modified food contains genes selected from sources with known allergenic effects, then it must be assumed that the novel gene product is allergenic unless proven otherwise. The current decision-tree approach which advocates the assessment of the binding of the novel protein with IgE from the blood serum of individuals who are allergic to the source of the donor genetic material followed, if necessary by skin testing and blinded oral food challenges, was considered adequate and essential. The assessment of any unintended effects on the allergenicity of the host material after a genetic modification with genes from other sources, whether allergenic or not, was not considered necessary except in circumstances where the genetic modification could be predicted to alter the protein content of the host product significantly.

When the genetically modified food contain genes from sources with no history of allergenicity, the current decision-tree approach relies primarily upon sequence homology comparisons to known allergens and the stability of the novel protein to digestion and processing. It is widely recognized that these two criteria alone may not be sufficient to assess the potential allergenicity of genetically modified foods containing genes from sources with no history of allergenicity.

The current criteria used to determine significant sequence similarity, a match of at least eight contiguous, identical amino acids (Metcalfe *et al*., 1996) has been criticized. Suggestions have been made that sequence similarity should instead require a match of a smaller number of contiguous, identical amino acids, perhaps as few as four amino acids. The use of a match of eight contiguous, identical amino acids appears to have some relevance based upon the minimum peptide length for a T cell-binding epitope[5] (Metcalfe *et al*., 1996). Also, it is recognized that the criterion cannot identify discontinuous or conformational epitopes that depend upon the tertiary

[5] Epitopes are groups of amino acids within proteins that can bind to either T cells (T cell epitopes) or IgE antibodies (IgE-binding epitopes). Epitopes can be either linear or conformational.

structure of the protein (Metcalfe *et al*., 1996). However, the stability of food allergens to heat processing argues for greater significance of linear, continuous epitopes for these particular allergens. International scientific consensus should be sought on the use of sequence homology in the assessment of the allergenicity of genetically modified foods.

The use of digestive stability appears to be a rather useful criterion in the assessment of the allergenicity of genetically modified foods. Simulated gastric and intestinal digestive models of mammalian digestion have been used to assess the digestive stability of known food allergens and proteins introduced into foods through genetic modification (Astwood *et al*., 1996). While the usefulness of this criterion is apparent, consensus is needed on the ideal protocols for assessment of digestive stability. It is recognized that novel proteins may exist that are stable to digestion but will not become allergens. Additional testing is needed to assess the allergenic potential of such proteins.

The desirability of the development of additional tests to assess the allergenic potential of foods containing genes from sources with no history of allergenicity has been widely expressed. Two additional tests seem to show some promise for addition to the decision-tree approach.

The level and site of expression of the novel protein is an important component of the assessment of allergenicity. Novel proteins expressed at comparatively low amounts in the food would have limited potential for allergic sensitization. Major food allergens are usually major proteins in commonly consumed foods. Thus, greater scrutiny should occur with genetically modified foods containing novel proteins at significant levels in the product. New proteins expressed in non-edible portions of plants are not a concern in terms of food allergy.

Consideration of the function of the novel protein should also form part of the decision-tree assessment of allergenic potential. Certain classes of proteins are well known allergens. For example, the 2S, high-methionine albumins from Brazil nut, walnut, sunflower seed and mustard are major allergens from those sources. Thus, other 2S, high-methionine albumins should be scrutinized very carefully for allergenic potential. Many of the pathogenesis-related proteins of plants display allergenic activity, therefore, the entire class of proteins would also merit close examination. International consensus should be sought on a list of functional proteins with allergenic potential. Certainly, other proteins not on such a list must also be evaluated but this aspect could be a useful part of an overall assessment strategy.

The potential for the use of animal models for the assessment of the allergenicity of genetically modified foods was also discussed. Unfortunately, reliable, well validated animal models for the assessment of the allergenicity of genetically modified foods do not presently exist, although further research on the development of animal models is encouraged.

Other attributes, such as molecular weight and degree of glycosylation, were also discussed. However, it was felt that these attributes were not sufficiently discriminatory to be very helpful.

The novel proteins present in genetically modified foods should also be evaluated for any possible role in the elicitation of gluten-sensitive enteropathy. Clearly, if the desired gene is obtained from wheat, rye, barley, oats, or related cereal grains, the possible role of the novel protein in provoking gluten-sensitive enteropathy must be carefully considered. Furthermore, if genetic modifications are conducted on these cereal grains, possible unintended effects on the gluten proteins should be considered. International consensus should be sought on an appropriate decision-tree approach to the assessment of the role of genetically modified foods and their novel proteins in gluten-sensitive enteropathy.

6.3.3 Reduction or Elimination of Allergens through Genetic Modification

Genetic modification offers the opportunity to decrease or eliminate the protein allergens that occur naturally in specific foods. An example is the development of a genetically modified rice variety developed through anti-sense technology, which dramatically reduced levels of the major rice allergen (Matsuda *et al.*, 1995). Further efforts of this type should be encouraged.

7. Conclusions

1. The Consultation agreed that the safety assessment of genetically modified foods requires an integrated and stepwise, case-by-case approach, which can be aided by a structured series of questions. A comparative approach focusing on the determination of similarities and differences between the genetically modified food and its conventional counterpart aids in the identification of potential safety and nutritional issues and is considered the most appropriate strategy for the safety and nutritional assessment of genetically modified foods.

2. The Consultation was of the view that there were presently no alternative strategies that would provide a better assurance of safety for genetically modified foods than the appropriate use of the concept of *substantial equivalence*. Nevertheless, it was agreed that some aspects of the steps in safety assessment process could be refined to keep abreast of developments in genetic modification technology. The concept of *substantial equivalence* was developed as a practical approach to the safety assessment of genetically modified foods. It should be seen as a key step in the safety assessment process although it is not a safety assessment in itself; it does not characterize hazard, rather it is used to structure the safety assessment of a genetically modified food relative to a conventional counterpart. The Consultation concluded that the application of the concept of *substantial equivalence* contributes to a robust safety assessment framework. The Consultation was satisfied with the approach used to assess the safety of the genetically modified foods that have been approved for commercial use.

3. The Consultation further agreed that the safety assessment of genetically modified foods requires methods to detect and evaluate the impact of unintended effects, such as the acquisition of new traits or loss of existing traits. The potential occurrence of unintended effects is not unique to the application of recombinant DNA techniques, but is also a general phenomenon in conventional breeding. Present approaches to detect unintended effects are based, in part, on the analysis of specific components (targeted approach). In order to increase the probability of detecting unintended effects, profiling techniques are considered as potentially useful alternatives (non-targeted approach). In order to assess the biological and safety relevance of an unintended effect, the genetically modified plant should first be compared to other conventional varieties and data on it compared to literature data. If the differences exceed natural variations, a nutritional and toxicological assessment is required. This may require an evaluation of specific components of the genetically modified food or of the whole food.

4. The Consultation considered the issue of long term effects from the consumption of genetically modified foods and noted that very little is known about the potential long term effects of any foods. In many cases, this is further confounded by wide genetic variability in the population, such that some individuals may have a greater predisposition to food-related effects. In this context, the Consultation acknowledged that for genetically modified foods, the pre-marketing safety assessment already gives assurance that the food is as safe as its conventional counterpart. Accordingly it was considered that the possibility of long term effects being specifically attributable to genetically modified foods would be highly unlikely. Furthermore, it was recognised that observational epidemiological studies would be unlikely to identify any such effects against a background of undesirable effects of conventional foods. Experimental studies, such as randomised controlled trials (RCTs), if properly designed and conducted, could be used to investigate the medium/long term effects of any foods, including genetically modified foods. Such studies could provide additional evidence for human safety, but would be difficult to conduct. In this respect, it is also important to recognise the wide variation in diets and dietary components from day to day and year to year.

5. The Consultation recognized that genetically modified foods with intentional nutritional effects may provide improved products for developed and developing countries. The change in nutrient levels in a particular crop plant may impact on overall dietary intake. In such cases, it is important to determine alterations in nutrient content and bioavailability, and their stability with time, processing and storage, as well as to monitor changes in dietary patterns as a result of the introduction of the genetically modified food and evaluate its potential effect on nutritional and health status of consumers. However, an assessment of the impact on nutritional status of consumers is important for all significant dietary changes and not specific to the introduction of genetically modified foods.

6. The Consultation agreed 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 foods should be evaluated for allergenicity on the basis of the decision-tree shown in Figure 1. 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.

7. The Consultation considered horizontal gene transfer from plants and plant products consumed as food to gut microorganisms or human cells as a rare possibility, but noted that it cannot be completely discounted. The most important consideration with respect to horizontal gene transfer is the consequence of a gene being transferred and expressed in transformed cells. An important example is the transfer of antimicrobial resistance genes, if it were to occur, from genetically modified foods to gut microorganisms. Important considerations for the assessment of the consequences of the transfer and expression of this gene in transformed cells would be the clinical and veterinary importance of the antibiotic in question, the levels of natural resistance and the availability of effective alternative therapies. In case of genes that confer resistance to drugs important for medical use, the possibility of transfer and expression of these genes is a risk that warrants their avoidance in the genome of widely disseminated genetically modified plants. The Consultation further noted that the antibiotic resistance markers currently used in genetically modified plants have been previously reviewed for safety. It concluded that there is no evidence that the markers currently in use pose a health risk to humans or domestic animals.

8. Recommendations

1. While the limitation of animal study methodology when used on whole food has been pointed out, the Consultation was of the view that in specific cases animal testing may be useful. It is recommended that further research and standardization should be initiated in this area.

2. The detection methods for unintended effects based on the analysis of specific components could be supplemented with alternative strategies, such as profiling techniques. These techniques are under development; it is recommended that these methods are further developed and validated. This will be especially important for more complex genetic modifications perhaps involving multiple between-species gene transfers.

3. It will be important to monitor changes in nutrient levels in foods from plants derived by conventional breeding and by genetic modification, and assess their effect on the nutritional status of the population. A number of future food products with specific nutritional changes will be especially relevant to the needs of developing countries, and efforts should be made to improve the dissemination of appropriate methodologies and capacity building in the developing world.

4. It is recommended that integration of nutritional and toxicological expertise needed for the evaluation of genetically modified foods be encouraged and facilitated. This will facilitate R&D in the area of genetic modification of plants and lead to an early identification of relevant safety and nutritional issues.

5. The Consultation encourages the use of alternative transformation technologies, if available and demonstrated to be safe, that do not result in antibiotic resistance genes in genetically modified foods. If further development of technology is required, additional research should be strongly encouraged.

6. It is recommended that consensus documents are developed to facilitate uniform application of the concept of *substantial equivalence*. These should include guidelines for appropriate design of field trials and the use of appropriate statistical methods to generate and analyse comparative data on genetically modified plants and their conventional counterparts.

7. Communication of the principles involved in the safety assessment of genetically modified foods should be improved. The Consultation concluded that the key message to be conveyed is that *substantial equivalence* is a concept used to identify similarities and differences between the genetically modified food and a comparator with a history of safe food use which in turn guides the safety assessment process.

8. 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.

9. The Consultation identified the following as the additional issues to be addressed in future FAO and WHO Consultations.

- Safety assessment specific to genetically modified micro-organisms

- Safety assessment specific to genetically modified animals (including fish)
- Safety assessment of functional food, including the nutritional aspects of the genetically modified foods
- Improved methodologies for the safety study of whole foods.
- The use of antibiotic resistance genes in plants and microorganisms for food production in relation to possible medical problems

9. References

1. Anderson, J. A. (1996): *Allergic reactions to foods.* Critical Reviews in Food Science and Nutrition **36**, S19-S38
2. Astwood, J. D., Leach, J. N. and Fuchs, R. L. (1996): *Stability of food allergens to digestion in vitro.* Nature Biotechnology **14**, 1269-1273
3. Beever, D. E. and Kemp, C. F. (2000): Safety issues associated with the DNA in animal feed derived from genetically modified crops. A review of scientific and regulatory procedures. Nutrition Abstracts Reviews, series B Livestock Feeds and Feeding **70**(3), 175-182
4. Bock, S. A. (1987): Prospective appraisal of complaints of adverse reactions to foods in children during the first three years of life. Paediatrics **79**, 683-688
5. Burks, A. W. and Sampson, H. (1993): *Food allergies in children.* Current Problems in Paediatrics **23**, 230-252
6. Commission of the European Communities (1997): Recommendations concerning the scientific aspects of the information necessary to support applications for the placing on the market of novel foods and novel food ingredients. Official Journal of the European Communities, L253/1-36
7. Doerfler, W. and Schubbert, R. (1997): *Fremde DNA im Säugersystem.* Deutsches Ärzteblatt **94,** 51-52
8. 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
9. Hefle, S. L., Nordlee, J. A. and Taylor, S. L. (1996): *Allergenic foods.* Critical Reviews in Food Science and Nutrition **36**, S69-S89
10. ILSI (1996): The safety assessment of novel foods: Guidelines prepared by ILSI Europe Novel Food Task Force. International Life Sciences Institute, European Branch, Brussels
11. Matsuda (1995): Rice allergenic protein and molecular-genetic approach for hypoallergenic rice. Bioscience, Biotechnology and Biochemistry **60,**1215-1221.
12. Mekori, Y. A. (1996): *Introduction to allergic disease.* Critical Reviews in Food Science and Nutrition **36**, S1-S18
13. Metcalfe, D. D., Astwood, J. D., Townsend, R., Sampson, H. A., Taylor, S. L. and Fuchs, R. L. (1996): *Assessment of the allergenic potential of foods derived from genetically engineered crop plants.* Critical Reviews in Food Science and Nutrition **36**, S165-S186
14. Nielsen, K. M.(1998), Bones, A. M., Smalla K., and van Elsas, J.D. (1998): *Horizontal gene transfer from transgenic plants to terrestrial bacteria - a rare event?* FEMS Microbiology Reviews **22** 79-103
15. OECD (1993): Safety evaluation of foods derived by modern biotechnology, Concepts and principles. Organisation for Economic Cooperation and Development, Paris
16. OECD (1996): *Food Safety Evaluation.* Report Workshop held in Oxford UK, Sept. 12-15, 1994. ISBN 92-64-14867-1.

17. Parker, S. L., Leznoff, A., Sussman, G. L., Tarlo, S. M. and Krondl, M. (1990): *Characteristics of patients with food-related complaints*. Journal of Allergy and Clinical Immunology **86**, 503-511

18. Sampson, H. A. (1990): *Immunologic mechanisms in adverse reactions to foods.* Immunology and Allergy Clinics of North America **11**, 701-706

19. Sampson, H. A. (1990): *Food Allergy*. Current Opinion in Immunology **2**, 542-547

20. Sampson, H. A. and Burkes, A. W. (1996): *Mechanisms of food allergy*. Annual Review of Nutrition **16**, 161-177

21. Schilter, B., Holzhäuser, D., Cavin, C. and Huggett, A. C. (1996): *An integrated in vivo and in vitro strategy to improve food safety evaluation*. Trends in Fd. Sci. Technol. **7**, 327-332

22. SCF (1997): Opinions on the Assessment of Novel Foods expressed 7 June 1996, 12/23 December 1996. European Commission DGIII/5915/97, Brussels

23. Schubbert, R., Hohlweg, U., Renz, D. and Doerfler, W (1998) On the fate of orally ingested foreign DNA in mice: chromosomal association and placental transfer to the fetus. Mol. Gen. Genet. **259**(6) 569-576

24. Taylor, S. L. (1997): Food from genetically modified organisms and potential for food allergy. Environ. Toxicol. Pharmacol. **4**:121-126

25. UK ACNFP (1995): *Advisory Committee on Novel Foods and Processes, Annual Report for 1994*. Ministry of Agriculture, Fisheries and Food, London

26. WHO (1987): *Principles for the Safety Assessment of Food Additives and Contaminants in Food*, Environmental Health Criteria 70. World Health Organization, Geneva

27. WHO (1991): Strategies for assessing the safety of foods produced by biotechnology, Report of a Joint FAO/WHO Consultation. World Health Organization, Geneva.

28. WHO (1993): Health aspects of marker genes in genetically modified plants, Report of a WHO Workshop. World Health Organization, Geneva

29. WHO (1995): Application of the principle of substantial equivalence to the safety evaluation of foods or food components from plants derived by modern biotechnology, Report of a WHO Workshop. World Health Organization, Geneva

30. Ye, X., Al-Babili, S., Klöti, A., Zhang, J., Lucca, P., Beyer, P., and Potrykus, I.: *Engineering the Provitamin A(ß-carotene) Biosynthetic Pathway into(Carotenoid-Free) Rice Endosperm*, Science **287**, 303-305

Annex 1: List of Participants

Experts

CHASSY, Bruce M., Head, Department of Food Science and Human Nutrition, UIUC, 905 S. Goodwin, 260 Bevier Hall, Urbana, Illinois 61801, U.S.A. Tel: +1 217 244 4497, Fax: +1 217 244 2455, E-mail: b-chassy@uiuc.edu

EWEN, Stanley W. B., Senior Lecturer, Department of Pathology, University of Aberdeen, Medical school, Foresterhill, Aberdeen, AB25 2ZD, Scotland. Tel: +44 1224 681 818 Ext. (5) 52845, Fax: +44 1224 663 002, E-mail: s.w.b.ewen@abdn.ac.uk

GODA, Yukihiro, Section Chief, Division of Foods, National Institute of Health Sciences, 1-18-1, Kamiyoga, Setagaya-ku, Tokyo, Japan. Tel: +81 3 3700 9397 (Direct), Fax: +81 3 3707 6950, E-mail: goda@nihs.go.jp

HADDADIN, Malik, Professor of Food Science, University of Jordan, Department of Nutrition and Food Technology, Jordan. Tel: +962 6 535 5000, Ext. 2676, Fax: +962 6 562 0641, E-mail: aborob_75@yahoo.com

KUIPER, Harry A.[6], Head, Department of Food Safety and Health 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 (*Chairperson*)

MARYANSKI, James, Biotechnology Coordinator, Center for Food Safety & Applied Nutrition, Food and Drug Administration, 200 C Street, SW – HFS-13, Washington, DC 20204, U.S.A. Tel: +1 202 205 4359, Fax: +1 202 401 2839, E-mail: james.maryanski@cfsan.fda.gov (*Rapporteur*)

MUNRO, Ian, Professor, Department of Nutritional Sciences, Faculty of Medicine, University of Toronto, Canada. Tel: +1 905 542 2900, Fax: +1 905 542 1011, E-mail: imunro@cantox.com

NUTTI, Marília Regini, Director, National Research Center for Food Technology, Brazilian Agricultural Research Corporation (EMBRAPA), Av. das Américas 29501, 23020-470 Guaratiba, Rio de Janeiro, RJ, Brazil. Tel: +55 21 410 1350, Fax: 55 21 410 1090, E-mail: marilia@ctaa.embrapa.br (*Vice-chairperson*)

PEDERSEN, Jan[7], Danish Veterinary and Food Administration, Institute of Food Safety and Toxicology, Division of Biochemical and Molecular Toxicology, Mørkhøj Bygade 19, 2860 Søborg, Denmark. Tel: +45 3395 6610, Fax: +45 3395 6001, E-mail: jp@fdir.dk

[6] author of Topic 5

[7] author of Topic 2

SCOTT, Fraser, Senior Scientist, Ottawa Hospital Research Institute; Associate Professor, Department of Medicine and Department of Biochemistry, Microbiology and Immunology, University of Ottawa, 501 Smyth Road, Ottawa, Ontario, K1H 8L6 Canada. Tel: +1 613 737 8929, Fax: +1 613 739 6189, E-mail: fscott@ottawahospital.on.ca

THOMSON, Jennifer[8], Professor and Head of the Department of Microbiology, University of Cape Town, PB Rondebosch 7701, South Africa. Tel: +27 21 650 3269, Fax: +27 21 689 7573, E-mail: Jat@molbiol.uct.ac.za(*Vice-rapporteur*)

VAVASOUR, Elizabeth, J., Chemical Health Hazard Assessment Division, Bureau of Chemical Safety, Food Directorate, Health Protection Branch, Health Canada, Sir Frederick Banting Building, P.L. 2204D1, Tunney's Pasture, Ottawa, Ontario, K1A 0L2 Canada. Tel: +1 613 957 1680, Fax: +1 613 957 1688, E-mail: elizabeth_vavasour@hc-sc.gc.ca

YANG, Xiaoguang, Professor, Institute of Nutrition and Food Hygiene, Chinese Academy of Preventive Medicine, 29 Nan Wei Road, Beijing 100050 P.R. China. Tel: +86 10 6317 1734(o), Fax: +86 10 6301 1875, E-mail: xgyang@public2.east.cn.net

Observers from International Organizations

KEARNS, Peter, Administrator, OECD, ENV/EHS, 2, rue André Pascal, 75775 Paris Cedex 16, France. Tel: +33 1 4524 1677, Fax: +33 1 4524 1675, E-mail: Peter.Kearns@OECD.ORG

GALLOWAY-MCLEAN, Kirsty, Biosafety/Scientific & Technical Information Officer of the Convention on Biological Diversity, 393 St-Jacques, Suite 300, Montréal, Québec, H2Y 1N9, Canada. Tel: +1 514 287 7039, Fax: +1 514 288 6588, E-mail: kirsty.mclean@biodiv.org

LANTIN, Manuel, Science Adviser, Consultative Group on International Agricultural Research (CGIAR), 1818H Street NW, Washington DC 20433, U.S.A. Tel: +1 202 473 8912, Fax: +1 202 473 8110, E-mail: mlantin@cgiar.org

STANTON, Gretchen, Counsellor, Agriculture and Commodities Division, World Trade Organization (WTO/OMC), Geneva, Switzerland. Tel: +41 22 739 5086, Fax: +41 22 739 5760, Email: gretchen.stanton@wto.org

Chairman of the Codex Ad Hoc Intergovernmental 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

[8] author of Topic 11

Secretariat

1) Authors of Working Papers (FAO/WHO Consultant)

BRUNNER, Eric[9], Senior lecturer in epidemiology, Dept. of Epidemiology & Public Health, University College, London, United Kingdom. Tel: +44 20 7679 1689/1267, Fax: +44 20 7813 0242/0280, E-mail: e.brunner@ucl.ac.uk

DOWNEY, Keith[10], CanoGlobe Consulting, 23 Simpson Crescent, Saskatoon, Saskatchewan S7H 3C5, Canada. Tel: +1 306 956 7298, Fax: +1 306 956 7247, E-mail: downeyk@em.agr.ca

ENGEL, Karl-Heinz[11], Technische Universität München, Lehrstuhl für Allgemeine Lebensmitteltechnologie, Am Forum 2, D-85350 Freising Weihenstephan, Germany. Tel: +49 8161 714 250, Fax: +49 8161 714 249, E-mail: k.h.engel@lrz.tu-muenchen.de

KUBO, Tomoaki[12], President & CEO, Orynova K.K., 700 Higashibara, Toyoda-cho, Iwata-gun, Shizuoka, 438-0802, Japan. Tel: +81 538 32 7111, Fax: +81 538 32 8700, E-mail: Tomoaki.Kubo@orynova.co.jp

OW, David[13], Plant Gene Expression Center, 800 Buchanan Street, USDA-ARS, Albany, CA, 94710 U.S.A. Tel: +1 510 559 5909, Fax: +1 510 559 5678, E-mail: Ow@pgec.ars.usda.gov

AL-BABILI, Salim[14], University of Freiburg, Freiburg, Germany. Tel: +49 761 203 2529, Fax: +49 761 203 2675, E-mail: albabili@uni-freiburg.de

TAYLOR, Steve[15], 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, U.S.A. Tel: +1 402 472 2833, Fax: +1 402 472 1693, E-mail: staylor2@unl.edu

TOMLINSON[16]**, Nick**, Food Standards Agency, Ergon House c/o Nobel House, 17 Smith Square, London SW1P 3JR, United Kingdom. Tel: +44 171 238 6377, Fax: +44 171 238 6382, E-mail: nick.tomlinson@foodstandards.gsi.gov.uk

WALKER, Ronald[17], 44 Ash Hill Road, Ash, Aldershot, GU12 6AB, United Kingdom. Tel: +44 1252 329 607, E-mail: r.walker@surrey.ac.uk

---

9 author of Topic 3
10 author of Topic 8
11 author of Topic 4
12 author of Topic 10
13 author of Topic 12
14 author of Topic 9
15 author of Topic 13
16 author of Topic 1
17 author of Topic 6

2) FAO/WHO Secretariat

BOUTRIF, Ezzeddine, Officer-in-Charge, Food Quality and Standards Service, Food and Nutrition Division, 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

SCHLUNDT, Jørgen, Coordinator, Programme of Food Safety, WHO, 20 Avenue Appia, 1211 Geneva 27, Switzerland. Tel: +41 22 791 3445, Fax: +41 22 791 4807, E-mail: schlundtj@who.ch

JONAS, David, Consultant, Wayborough Bungalow, Wayborough Hill, Minster, Ramsgate CT12 4HR, United Kingdom. Tel: +41 1843 821 745, Fax: +41 1843 822 566, E-mail: DavidJonas1@compuserve.com (*WHO Consultant*)

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 (*FAO Joint Secretary*)

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.ch (*WHO Joint Secretary*)

WEISELL, Robert, Nutrition Officer, Nutrition Planning, Assessment and Evaluation Service, FAO, Viale delle Terme di Caracalla, 00100 Rome, Italy. Tel: +39 06 570 53982, Fax: +39 06 570 54593, E-mail: robert.weisell@fao.org

Annex 2: List of Documents[18]

Biotech 00/01	Provisional Agenda and Timetable
Biotech 00/02	Provisional Annotated Agenda
Biotech 00/03	Topic 1: The Concept of Substantial Equivalence, its Historical Development and Current Use
Biotech 00/04	Topic 2: Application of Substantial Equivalence; Data Collection and Analysis
Biotech 00/05	Topic 3: Limitations of Substantial Equivalence Regarding the Assessment of Foods Derived from Biotechnology
Biotech 00/06	Topic 4: Unpredictable Effect of Genetic Modification
Biotech 00/07	Topic 5: Profiling Techniques to Identify Differences between Foods Derived from Biotechnology and their Counterparts
Biotech 00/08	Topic 6: Safety Testing of Food Additives and Contaminants and the Long Term Evaluation of Foods Produced by Biotechnology
Biotech 00/09	Topic 7: Nutritional Implication of Biotechnology (*Not available*)
Biotech 00/10	Topic 8: Evaluation of Foods with Altered Major Components
Biotech 00/11	Topic 9: Evaluation of Foods that have been Nutritionally Enhanced by Biotechnology
Biotech 00/12	Topic 10: Potential of Foods from which Unfavourable Components have been Removed.
Biotech 00/13	Topic 11: Gene Transfer: Mechanisms and Food Safety Risks
Biotech 00/14	Topic 12: Marker Genes
Biotech 00/15	Topic 13: Allergenicity

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

Annex 3: Reply to the Questions from the Codex *ad hoc* Intergovernmental Task Force

1) What overarching scientific principles should be applied to the safety and nutritional assessment?

Experience throughout the world has led to the identification of a number of common scientific principles currently used in safety and nutritional assessment.

The existing food supply has a long history of safe use, even though some foods are not safe for some individuals and many foods contain substances that would present health concerns if they were present above accepted levels. Most foods derived using recombinant DNA techniques are obtained from traditional crops that have usually been modified to exhibit one or a few well-defined traits. The knowledge and experience gained in the use of traditional crops is an important component in the safety assessment of foods derived from such plants.

Safety assessment of whole foods and many complex food ingredients requires use of an approach that differs from the strategy used to assess safety of single, well-defined chemicals, such as food additives, pesticides and contaminants. The approach for whole foods is case-by-case, based on an evaluation of multi-disciplinary data and information, that is derived from, as appropriate, but is not limited to, agronomic, genetic, molecular biological, nutritional, toxicological and chemical properties. Toxicology testing in animals is not routinely employed, but when necessary based on an assessment of available data and information, tests should be designed to address specific issues.

The following issues are some of the main points considered in the evaluation: the new gene, the new protein and other food components, taking into account both intended and unintended changes in the food and steps to reduce the likelihood of adverse, unexpected effects. In specific cases, additional effects (such as antibiotic resistance) may be evaluated.

Genetically modified foods and conventional foods have many characteristics in common, and in many cases, the new food or food ingredient will be nutritionally equivalent to its conventional counterpart.

Analytical methods traditionally applied in the evaluation of food constituents such as total protein, fat, ash, fibre and micronutrients may need to be augmented with additional analyses using profiling methods to identify unexpected effects and modified nutrient profiles which may impact dietary intake and health.

Because of the potential for broad changes in nutrient levels and interactions with other nutrients as well as unexpected effects, it may be necessary in certain instances to undertake feeding tests in animals to determine outcomes that result from changes in nutrient profiles and nutrient bioavailability. Nutritional modifications which are within normal ranges of nutrient variation might require a less extensive evaluation than those outside normal ranges.

The data and information should be of a quality and quantity that would withstand scientific peer review. Safety assessment is designed to identify information on the nature and the severity of any hazards that may be present, allowing appropriate management methods to be defined.

In conclusion, safety assessment of food and food ingredients obtained using recombinant DNA techniques does not require new scientific principles or methodology. Similar principles for the assessment of the safety and wholesomeness of genetically modified foods should be

applied as practised for conventional foods. Depending on the characteristics of the genetic modifications, specific safety and nutritional aspects are assessed.

2) What is the role, and what are the limitations, of substantial equivalence in the safety and nutritional assessment? Are there alternative strategies to substantial equivalence that should be used for the safety and nutritional assessment?

The concept of *substantial equivalence* is well established as an important component in safety assessment, and has been elaborated in several international reports. It is based on the idea that an existing organism (plant) used as food, or as a source of food, can serve as the basis for comparison when assessing the safety for human consumption of a food or a food component that has been modified or is new. There is a broad consensus that *substantial equivalence* is of value in safety assessment.

Application of the concept of *substantial equivalence* may lead to the identification of similarities and defined differences in the food and food ingredients. Further safety assessment will be focused on establishing the safety of the differences in the new product such that safety of the food or food ingredient can be established, relative to its comparator. The safety assessment carried out in this way does not provide an absolute safety warrant for the new product.

Another aspect of the concept of *substantial equivalence* is that it can only be applied where there is a suitable comparator. This requires that sufficient data is available or can be generated for the comparator. Where there is no comparator, *substantial equivalence* cannot be used to assess safety. In such cases, safety testing will be required based on the properties of the food concerned.

Current strategies for assessing the safety of foods derived from genetically modified plants are considered appropriate. There are presently no alternative strategies that would provide a better assurance of safety for genetically modified foods than the appropriate use of the concept of *substantial equivalence*. However, some aspects of the steps in safety assessment process could be refined to keep abreast of developments in genetic modification technology. Methodologies, such as profiling techniques, offer a means of providing a more detailed analytical comparison. However, much more developmental work would be necessary before such methods could be validated.

3) What scientific approach can be used to monitor and assess possible long-term health effects or unintended/unexpected adverse effects?

The Consultation considered that the methodologies for safety evaluation elaborated in the report are adequate to detect and evaluate any possible long-term effects of genetically modified foods.

The Consultation considered the issue of long-term effects from the consumption of genetically modified foods and noted that very little is known about the potential long-term effects of any foods. In many cases, this is further confounded by wide genetic variability in the population, such that some individuals may have a greater predisposition to food-related effects.

Against this background, the Consultation acknowledged that for genetically modified foods, the pre-marketing safety assessment already gives assurance that the food is as safe as its

conventional counterpart. Accordingly it was considered that the possibility of long-term effects being specifically attributable to genetically modified foods would be highly unlikely.

An important aspect of the safety assessment is a consideration of the nature of the introduced gene product. Where there is no history of consumption of the introduced gene product or of the food, a 90-day study will probably be indicated. If such studies show evidence suggesting possible long-term effects, e.g. evidence of cell proliferation, further long-term studies would need to be considered if the development of the product was to continue.

The Consultation was of the view that monitoring to establish links between diet and disease is desirable. However, many chronic health effects are multifactorial and it was recognised that observational epidemiological studies would be unlikely to identify any such effects against a background of undesirable effects of conventional foods. Experimental studies, such as randomised controlled trials (RCTs), if properly designed and conducted, could be used to investigate the medium/long term effects of any foods, including genetically modified foods. Such studies could provide additional evidence for human safety, but would be difficult to conduct. In this respect, it is also important to recognise the wide variation in diets from day to day and year to year.

The same problems apply to the detection of potential long-term beneficial health effects. Nevertheless, it was recognised that genetically modified foods intended to produce nutritional effects are under development for use in developed and developing countries. In such cases, a change in nutrient levels in a particular crop plant may impact overall dietary intake and it would be important to monitor changes in nutrient levels in such foods and evaluate their potential effect on nutritional and health status.

The potential occurrence of unintended effects is not specific for the application of recombinant DNA techniques, rather it is an inherent and general phenomenon in conventional breeding. One of the approaches to cope with this problem is to select and discard plants with unusual and undesired phenotypic and agronomic parameters already at an early stage. The practice of consecutive back-crossing is also a major procedure used to eliminate unintended effects. Only in rare cases are these approaches accompanied by analytical screening of defined constituents.

Unintended effects due to genetic modification may be subdivided into two groups: those which are "predictable" based on metabolic connections to the intended effect or knowledge of the site of insertion and those which are “unexpected”. Due to the increased precision of genetic modification compared to conventional breeding, it may become easier to predict pathways likely to be influenced by unintended effects.

The comparator used to detect unintended effects should ideally be the near isogenic parental line grown under identical conditions. In practice, this may not be feasible at all times, in which case a line as close as possible should be chosen. The resulting natural variation should be taken into account in assessing the statistical significance of the unintended effect.

Where statistically significant unintended differences are observed, their biological significance should be assessed. This may be assisted by knowledge of the mechanisms leading to the changes. In order to assess the biological and safety relevance of an unintended effect, data on the genetically modified plant should be compared to data on other conventional varieties and literature data. If the differences exceed natural variations in traditional food crops, further assessment is required.

Present approaches to assess possible unintended effects are based, in part, on the analysis of specific components (targeted approach). In order to increase the probability of detecting

unintended effects, profiling techniques are considered as useful alternatives (non-targeted approach). Profiling techniques are used at different level e.g. genomics, proteomics and metabolomics.

In the future, genetic modifications of plants are likely to be more complex perhaps involving multiple between-species transfers and this may lead to an increased chance of unintended effects. In such cases, profiling techniques may contribute to the detection of differences in a more extensive way than targeted chemical analysis but they are not yet fully developed and have certain limitations. Having detected differences using profiling techniques, their safety implications of such difficulties will still need to be considered.

4) What scientific approach can be used to assess the potential allergenicity?

An assessment of the potential allergenicity should be made for all genetically modified foods. In the assessment, the novel proteins resulting from the inserted gene should be the focus of the investigation in most cases.

An assessment of the potential allergenicity of the genetically modified food should be conducted in all cases. Possible enhancement of the inherent allergenicity of the host plant food should also be included in the assessment only when the intended effect of the genetic modification involves a significant alteration of the protein content of the food product derived from the host plant.

A decision-tree strategy should be applied in the assessment of the potential allergenicity of the novel protein(s). When the transferred gene is obtained from a source with a known history of allergenicity, the assessment should focus initially upon the immunochemical reactivity of the newly introduced protein with IgE from the blood serum of individuals with known allergies to the source of the transferred genetic material. Where necessary (in cases where no evidence of immunochemical reactivity is obtained), skin tests with extracts of the novel protein and blinded oral food challenges with the genetically modified food should be conducted on individuals with known allergies to the source of the transferred genetic material to provide confirmation that the novel protein is not allergenic. This series of tests provides adequate evidence regarding the allergenicity (or lack thereof) of novel proteins expressed by genes obtained from known allergenic sources.

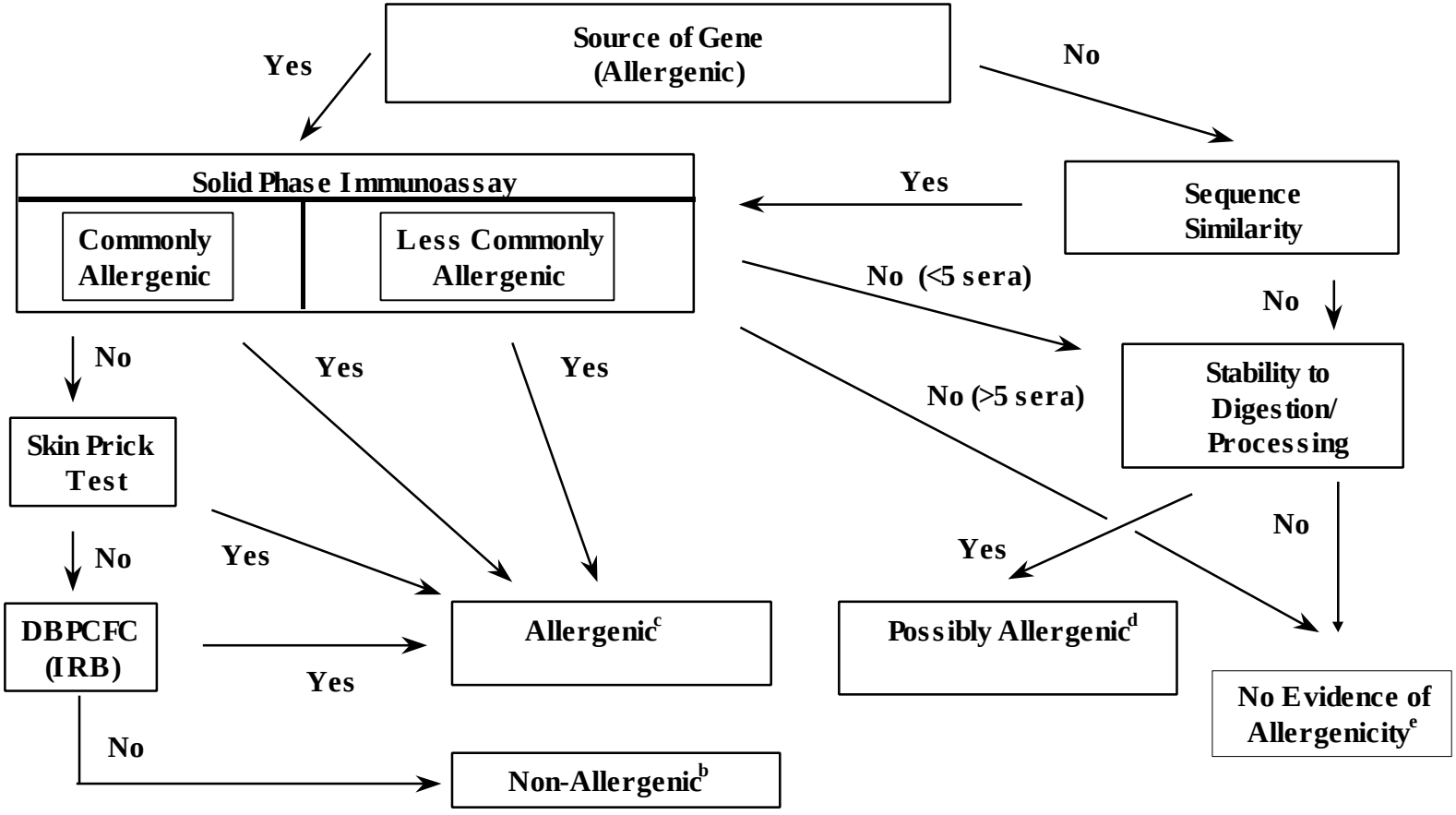
The decision-tree approach should rely upon various criteria used in combination (since no single criterion is sufficiently predictive). The current criteria include the sequence homology of the newly introduced protein to known allergens, the immunochemical reactivity of the newly introduced protein with IgE from blood serum of appropriate, allergic individuals when sequence homology is found, and the stability of the novel protein to digestion in gastric and intestinal model systems. This Consultation suggests that the incorporation of two additional criteria to the decision-tree approach when the genetic material is not known to be allergenic might be useful. The level and site of expression of the novel protein and the functional properties of the novel protein should be considered for addition to the list. These criteria taken together offer reasonable evidence that the novel protein is not allergenic, is not cross-reactive with known allergens, and has limited potential to become a food allergen. However, the development of additional criteria could offer additional confidence in the decision-tree approach. In particular, this Consultation advocated continued research on the development of a well-validated animal models for the assessment of the potential allergenicity of novel proteins from genetically modified foods. The Consultation also advocated additional research to identify allergenic proteins in food and to determine their protein sequences.

5) What scientific approach can be used to assess the possible risks arising from the use of antibiotic resistance marker genes in plants and microorganisms?

In genetically modified plants, the product of an antibiotic resistance gene must be subjected to standard safety assessments as would be performed on any other introduced gene product. Thus the product of the antibiotic resistance gene must be assessed for toxicity and potential allergenicity.

Where antibiotic resistance marker genes are present in plants or microorganisms, the possibility of transfer of the genes to pathogenic microorganisms and possible clinical implications must be considered. Horizontal gene transfer from plants and plant products consumed as food to gut microorganisms or human cells is considered as a rare possibility, but cannot be completely discounted. The most important consideration with respect to horizontal gene transfer is the consequence of a gene being transferred and expressed in transformed cells. An important example is the transfer of antimicrobial resistance genes, if it were to occur, from genetically modified foods to gut microorganisms. Important considerations for the assessment of the consequences of the transfer and expression of this gene in transformed cells would be the clinical and veterinary importance of the antibiotic in question, the levels of natural resistance and the availability of effective alternative therapies. In general, antibiotic resistance genes used in food production that encode resistance to clinically important antibiotics should not be present in widely disseminated genetically modified organism or foods and food ingredients.

Assessment of the Allergenic Potential of Foods Derived From Genetically Modified Crop Plants



Footnotes to Figure

(a) The figure was adapted from decision-tree approach developed by International Food Biotechnology Council and Allergy and Immunology 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.