

Genetically modified crops and allergenicity

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Among the concerns surrounding genetically modified crops has been the possibility of expressing within a plant new proteins that may be allergenic. Using available technology, practical approaches have been adopted to help prevent the creation of foods that are allergenic.

Genetically modified (GM) plants are produced by altering the DNA of the plant genome, mitochondria or chloroplasts through the introduction, rearrangement or removal of DNA. These manipulations involve such methods as infection with recombinant vectors, electroporation or particle bombardment. The resulting GM crops offer improved pest and disease resistance; higher yields; superior flavor, appearance and nutrition; tolerance of specific herbicides; and reduced requirements for fertilizer or water¹. The US National Center for Food and Agricultural Policy reports that genetically transformed corn raised yields in the United States by 47 million bushels on 4 million acres during 1997, the year of a corn borer insect infestation, and by 60 million bushels on 14 million acres in 1998 (ref. 2). Similarly, cotton biotechnology resulted in 5 million fewer acres of pesticide applications while increasing harvests by 85 million pounds. Additional benefits include reductions in synthetic pesticides and production costs, conservation of arable forests and wetlands, and improved food safety resulting from reduced amounts of pathogenesis-related toxins produced in plants in response to stress, some of which are allergens.

By 2003, the GM crops of soybean, cotton, maize and canola were planted on approximately 67.7 million hectares globally³. With estimates indicating that the world population will climb above 7.5 billion by the year 2020 and 9 billion by 2050 (ref. 4), the

benefits of GM crops seem critical to producing the quantities of crops necessary for worldwide nourishment. Importantly, the regulation of these GM crops in the United States is based on a legal notification process and a recommended safety analysis developed by the US Food and Drug Administration, presented in the *Federal Register*, Vol. 57, No. 104, May 29, 1992.

Despite the benefits of GM crops, the potential health hazards of each genetically transformed food, including the risk of allergenicity, need to be understood and approaches developed to ensure that no harm will result from intended uses. This caution has led to strategies to monitor transformed crops for allergenic potential before release. Such approaches are based on what is known about the pathogenesis of food hypersensitivity reactions and the characteristics of food allergens. In the process, immunologists, allergists and food technologists have been challenged to translate scientific and clinical observations into practical approaches to prevent the creation and marketing of new allergenic foods.

Nature of food allergy

The majority of biotechnology applications have resulted in the expression of new proteins in the GM crop. Although relatively few proteins are allergens, most allergens are proteins. Thus, the allergenic potential of foods, especially those that contain transformed proteins and to which humans have not previously been exposed, is of concern. Antibody (IgE)-mediated reactions, which include hives, asthma and anaphylaxis (**Box 1**), are the basis for most allergic reactions to food. These responses occur after the release of chemical mediators from mast cells and basophils as a

result of interactions between food proteins and specific IgE molecules on the surfaces of these effector cells.

Allergenic foods typically contain multiple allergens, and these allergens are designated using the first three letters of the genus name, the first letter of the species name and a number indicating the order of designation. Major allergens are usually defined by the demonstration that more than 50% of patients allergic to that food react to a particular protein. Examples of species and allergen designations from among the foods that appear to cause nearly 90% of reported food allergy reactions include milk (cattle, *Bos domesticus*; *Bos d 4–8*), soy (soybean, *Glycine max*; *Gly m1A* and *Gly m1B*), peanut (*Arachis hypogaea*; *Ara h 1–3*), crustaceans (for example, Indian shrimp, *Penaeus indicus*; *Pen I 1*), fish (for example, cod, *Gadus callarias*; *Gad c 1*), egg (chicken, *Gallus domesticus*; *Gal d 1–4*) and tree nuts such as Brazil nut (*Bertholletia escelsa*; *Ber e 1*) and walnut (*Juglans regia*).

Reports evaluating food allergy in randomly selected adults from Australia (26–50 years of age) and from the 1998 European Community Respiratory Health Survey (20–45 years of age) demonstrated probable IgE-mediated (via skin prick test) food allergy prevalence of less than 1.5%^{5,6}. This frequency is in reasonable agreement with reports that approximated the prevalence of food allergies in the United States and the United Kingdom as near 1.3% and 1.5%, respectively⁷. The probable prevalence of food allergy in children three years of age or younger is estimated to be considerably higher (6%)⁸. More recently, studies of European children suggest that the frequency of IgE-mediated food allergy may range up to 10% at one year of age and 7% at two years and then decreases to 3% by five to six years^{9–11}. The loss

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BOX 1 IMMUNE-MEDIATED ADVERSE REACTIONS TO FOODS

1. IgE-mediated (immediate)

- Urticaria and angioedema
- Rhinoconjunctivitis, asthma
- Oral allergy syndrome
- Nausea, colic, vomiting, diarrhea
- Anaphylaxis

2. Non-IgE-mediated (delayed)

- Food-induced enterocolitic syndromes
- Celiac disease

3. Overlap

- Atopic dermatitis
- Allergic eosinophilic gastroenteritis

4. Controversial

- Migraine headaches
- Irritable bowel syndrome

of sensitivities to food allergens (particularly those from milk and egg) by older children is not uncommon, whereas individuals with allergies to nuts or seafood seem less likely to lose their clinical reactivity¹².

Characteristics of food allergens

The pathophysiologic mechanisms involved in the development of food hypersensitivity are incompletely understood. Under normal circumstances in most individuals, ingested food proteins are not allergenic and thus do not elicit allergic immune responses. This absence of reactivity may be due to immune suppression or tolerance. However, a deficiency of such processes in some individuals, usually genetically based, results in their immune system recognizing some foreign proteins as allergenic, thus leading to food-induced allergic reactions.

Although hundreds of proteins have been identified as allergens (including inhaled allergens) and their amino acid sequences have been characterized and incorporated into comprehensive allergen databases¹³, the precise features of a protein that make it allergenic remain elusive. The more common food allergens are generally water-soluble proteins that have a reasonable degree of glycosylation and range in size from 10 to 70 kDa. Many allergens seem to be storage proteins and as such are present in large amounts. However, exposure to protein is a necessary part of allergic sensi-

zation, and there are numerous food allergens that are present in relatively small amounts, thus demonstrating the importance of potency versus exposure dose. Some food allergens have similarity to, and thus can be classified as, pathogenesis-related proteins¹⁴. This class of proteins is important in the plant's defense mechanism and seems to be more abundant in situations of environmental stress, physical damage or infestation. A number of food allergens are also stable and resistant to heat (that is, cooking) and digestive processes¹⁵. Conversely, some food allergens are unstable, for example allergens from apples and milk that have been shown to be labile¹⁶. Furthermore, denaturation of proteins through digestion or heat processing may enhance the allergic properties of foods, as has been suggested to occur for peanuts¹⁷. Despite these general commonalities of known allergens, the exceptions are sufficient enough that predicting the allergenicity potential of proteins based solely on these characteristics is inadequate.

Predicting potential for allergenicity

Prediction of the allergenicity potential of modified foods is largely based on whether the source of the newly expressed protein is a plant known to produce a food allergen and on the characteristics of the newly expressed protein. Current approaches have deficiencies that make the evaluation of a poorly characterized protein problematic. A decision tree consisting of a defined sequence of steps in allergenicity assessment was first proposed in 1996 (Fig. 1). This evaluation process relies upon an assessment of the gene source, an amino acid sequence homology comparison to known allergens, and assessments of serum IgE reactivity from known allergic patients and protein stability in the presence of enzyme(s)⁴. This strategy includes features used to identify a methionine-rich 2S albumin from Brazil nuts, which was transfected into soybeans to address methionine deficiency, but was found to be an allergen. The protein was later determined to be a major Brazil nut allergen, now known as *Ber e 1* (ref. 18), resulting in the termination of further development of this crop.

There are, however, limitations inherent to the proposed assessment of allergenic potential that relate to testing foods using allergic sera and relating to the predictive value of stability assays. Although there is apparent value in screening foods against sera containing allergen-specific IgE to assure that the GM food does not express a known food allergen, it is difficult to obtain clinically verified IgE samples from patients allergic to specific foods in sufficient numbers to be useful, particularly in the case of less common food allergens. In

fact, even through this strategy is preserved in subsequent assessment approaches, most transferred genes are not taken from known allergenic source(s), and thus in practice this approach would seldom be used. Similarly, the 1996 decision tree also included approaches to measure IgE reactivity *in vivo* through skin testing or food challenge. This has led to ethical concerns related to human studies of this sort, where the subjects would not directly benefit from the research results.

As noted, a number of food allergens seem relatively resistant to heat and protease degradation¹⁵. Thus, heat stability and enzyme digestion (for example, with pepsin or trypsin) characteristics were used as a means to help identify potential allergens. Such protein stability may be an indication of the integrity of proteins passing through the stomach or of the fact that their allergenicity is determined by linear epitopes rather than conformational epitopes (which are less likely to survive digestion). Stability characteristics may also reflect a protein's behavior during enzymatic alteration by antigen-processing cells. However, in studies that compare allergens and non-allergens from different protein classes, including storage and contractile proteins, enzymes and lectins, digestion data have not uniformly demonstrated that food allergens are consistently more stable than non-allergens or proteins of unknown allergenicity¹⁹. A final limitation of an enzyme digestibility assay is a lack of standardization. Ratios of protein to enzyme, as well as thresholds of 'stability' in terms of time until degradation and techniques for digest detection, have large implications for the interpretation of digestibility data.

Evolution of allergenicity assessment

In 2001, a revised decision tree approach was set forth by the Food and Agricultural Organization/World Health Organization (FAO/WHO) of the United Nations, which convened a group of experts from academia, industry and government with expertise in protein chemistry, animal models of safety assessment, immunology, food processing and food labeling to advance the strategy for allergy safety assessment of genetically transformed foods (Fig. 2; ref. 20). The result was a modified decision tree that added features such as IgE reactivity with plants that are broadly related to the gene source of the transferred DNA, as well as an increased emphasis on animal models and expression levels of the new protein to push the boundaries of safety assessment. The 2001 decision tree also increased the rigor in the evaluation of amino acid sequence homology from 8 amino acids down to 6 amino acids,

along with a 35% identity match over any 80 or more contiguous amino acids throughout the sequence of the protein.

A number of studies have since reviewed the amino acid homology recommendations and found them problematic, largely because such searches lack sufficient predictive value. For instance, 41 of 50 randomly chosen proteins from maize, which itself is rarely allergenic, matched at least one allergen or putative allergen using the criteria of a match of 6 contiguous amino acids²¹, and 9 of 50 shared greater than 35% identity to an allergen over any segment of at least 80 amino acids. What does seem to be true, and logical, is that the more identity a given protein has with a known allergen, the more likely the protein is to be allergenic. For example, proteins sharing more than 70% overall identities often show serologic cross-reactivity²². Such observations suggest that refined sequence and antigenicity motif recognition algorithms are needed, although it seems unlikely that any computer search and prediction tool will approach 100% accuracy in determining whether a protein is likely to be or to become an allergen. Thus, the goal of informatics should be the identification of proteins that may be allergenic, which will then require further evaluation.

Although reliable animal models have yet to be identified, the 2001 FAO/WHO report on allergenicity assessment of foods derived from biotechnology encouraged the use of animal studies to help evaluate allergenicity potential. This has prompted subsequent consultations and workshops organized to advance the science of animal allergenicity models (such as the ILSI Protein Allergenicity Technical Committee, Workshop on Animal Models to Detect Allergenicity to Foods and Genetically Modified Products (Health Canada) and Assessment of the Allergenic Potential of Genetically Modified Foods (US Environmental Protection Agency, Food and Drug Administration and National Institutes of Health)). One view deriving from these meetings is that an appropriate animal model designed for hazard identification of allergenicity potential should do so in the context of specific IgE production. However, given the differences in immune responses between various animal species and man, this model may not necessarily be useful. Further, the model need not necessarily mimic human exposure mode (that is, oral) or clinical response(s), nor should it demonstrate significant IgE production after exposure to non-allergens. As a hazard identification model, a model needs to accurately reflect the allergen potency of known allergens in an order that is similar to that reported for humans (peanut > egg > milk > potato) as

measured by some parameter of response. A number of laboratory species are under critical evaluation. These include the Brown Norway rat, a high IgE-responding rat strain that is thought to mimic the genetic phenotype of atopic humans; the BALB/c mouse, another IgE-responding rodent; and allergic models using 'atopic' dogs and neonatal swine.

Additional approaches to assure food safety that have been discussed in support of allergenicity assessment include protein concentrations and post-market surveillance. Although the allergenicity of a protein is likely to be more important than the exposure dose, the upper limit of abundance of transfected gene product(s) would be an informative piece of evidence in determining the likely sensitization potential. Unfortunately, insufficient data exist to support threshold values for the amounts of allergens required for sensitization, although more data are available on the amount required to elicit an allergic response. Furthermore, the amounts of transfected gene product might only be useful to help determine risk once a protein is deemed allergenic. However, it seems that doses ranging from micrograms to several milligrams are necessary to elicit a clinical allergic reaction to the more common allergens, such as peanut, milk and egg, in subjects most sensitive to one of these allergens²³.

Although it does not provide an *a priori* evaluation of allergenicity potential, post-market surveillance may provide safety information regarding long-term consumption and exposure. One commonly proposed strategy involves adverse event reporting. A cluster of adverse reports would subsequently need to be confirmed through IgE reactivity tests to

validate the causal relationship between exposure to the offending food ingredient and self-reported effects. Assessment of this approach suggests that adverse event reporting may not detect a modest allergic outbreak against the background of allergic reactions caused by conventional foods. Post-market surveillance may only be useful if it can be accompanied by the daunting task of 'traceability' of genetically transformed crop.

Summary guidance

Because of the complexity of the approaches outlined above, the Codex Alimentarius Commission (CODEX), an FAO/WHO joint organization that devises and standardizes international food codes, determined an approach to safety assessment of GM foods that incorporates simultaneous evaluations using a number of methodologies relating to the approaches reviewed above. These approaches are to be modified as the science for each parameter advances to make them trustworthy predictors of allergenicity²⁴.

As no single approach to evaluating allergenicity potential absolutely determines the allergenic potential of a food protein, the best current approach seems to be to use a combination of techniques in an organized and consistent manner, to provide an accurate safety assessment. Animal models may eventually serve as the most informative approach for identifying the allergenic potential of genetically modified food, and efforts to refine these models continue. For now, IgE reactivity in human patients remains an available direct approach in the evaluation of direct immune recognition, but only if the protein has

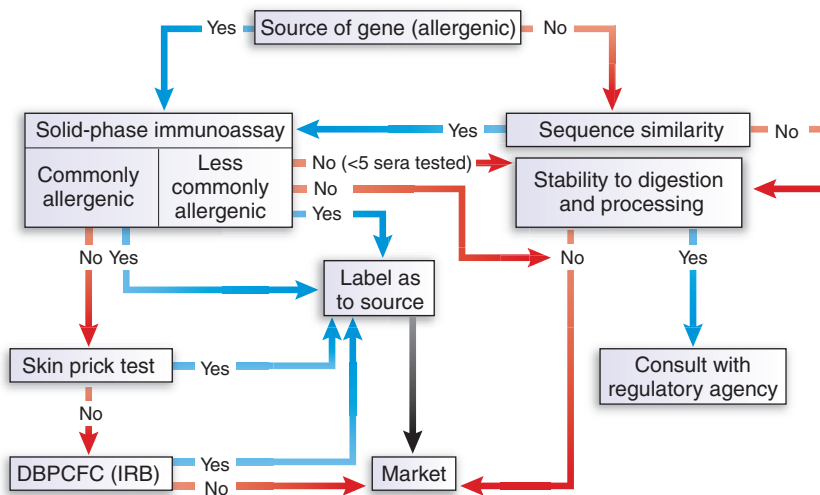


Figure 1 Proposed step-by-step 'decision tree' assessment for allergenicity of GM crops. 1996 decision tree advanced by an Institute of Food Technologists expert panel on biotechnology and foods.

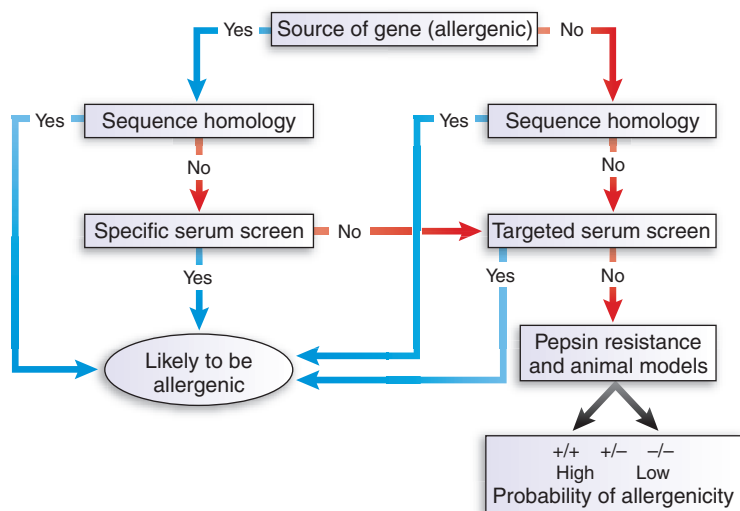


Figure 2 Proposed step-by-step 'decision tree' assessment for allergenicity of GM crops. 2001 revised decision tree set forth by a FAO/WHO consultancy.

characteristics homologous to those of another allergen. In practice, allergen-homologous proteins will more often than not be excluded from R&D efforts after bioinformatic analyses (such as amino acid sequence comparison). An additional problem with testing reactivity to patient IgE is the need for appropriate and clinically well-defined serum samples. Although the development of an all-inclusive serum bank is frequently discussed, the collection, characterization and maintenance of such a bank represents a substantial commitment of resources.

The information provided by amino acid sequence comparisons requires that this approach receive attention as well. To advance a consistent process of allergenicity assessment, however, an agreed-upon, standardized bioinformatics approach must be used. Amino acid homology screening is conducted early in product development and,

it appears, should remain a step in allergenicity assessment. It should not be used as the sole approach, however, because sequence homology as it relates to allergen characteristics remains to be well understood in the context of hazard as well as risk. Protein stability data have frequently been used in combination with bioinformatics as determinants of allergenicity potential, and the utility of this technique is based on the tenet that protein stability is necessary for mucosal exposure to the immune system. As such, this approach may continue to be useful in the assessment of protein exposure, once the hazard potential for allergenicity potential has been elucidated, and thus should be standardized to provide consistent results among investigators. Methods such as proteomics may provide useful data in regard to exposure, while being of value in providing insight into the overall compositional equivalency.

A consistent, logically ordered approach that incorporates multiple techniques would currently seem to provide the most effective safety assessment of allergenic potential of GM crops.

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