

Approaches to the Safety Assessment of Novel Foods and Novel Gene Products

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INTRODUCTION

The safety of genetically modified foods should be considered and assessed at all stages of the developmental process. The introduction of foods produced through agricultural biotechnology has generated considerable concern among certain consumers in many countries. The safety of these novel products has often been called into question during the ongoing public debate. So, safety assessment of these novel foods is absolutely crucial to their acceptance by consumers. Safety assessment must be a consideration at all stages of the development of a genetically modified food: gene discovery, line selection, and product advancement to commercialization. This article focuses on the role that safety assessment plays in all three of those stages. Particular emphasis is placed upon allergenicity assessment, but we must recognize that allergenicity is but one factor to consider in a comprehensive safety assessment approach.

In many countries, the safety assessment for genetically modified foods is mandatory and a review of the safety assessment data is conducted by the regulatory authorities. Some concern has arisen because the safety assessment process in the United States has been voluntary, but the U.S. Food & Drug Administration has announced its intent to create a mandatory safety assessment for genetically modified foods. However, the genetically modified foods allowed on the U.S. market thus far have been subjected to a de facto mandatory safety assessment system because governmental regulatory agencies have reviewed the available safety assessment information.

Thus far, all the genetically modified products brought into the marketplace have been subjected to such an intensive safety assessment. The safety assessment data have been reviewed by regulatory authorities around the

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world, and the current generation of genetically modified products is quite safe for human and feed animal consumption.

GENE DISCOVERY STAGE

In the gene discovery stage, a product concept is developed, and scientists screen and select genes that might fulfill that concept. The safety assessment process ideally should begin at the gene discovery stage. If any questions arise at this stage, these questions must be answered either immediately or later in the developmental process. Often careful selection of genes avoids many of the more difficult safety assessment issues. The selection of suitable genes should take into account various factors including the source of the gene, any previous consumer exposure to this source material, the history of safe use of the source material, the gene, and the product of the gene, and any ethical issues that might occur. Environmental and ecological concerns also should be considered at this early stage, although these issues are beyond the scope of this particular article. If unacceptable safety risks are identified at this stage, the concept probably should be abandoned, especially if the identified risks cannot be completely and clearly evaluated before commercialization.

The initial safety assessment at the gene discovery phase is important because it draws attention to concerns and highlights questions that must be effectively addressed later provided the concept is not abandoned at this point. For example, concerns at the gene discovery stage might regard such issues as the allergenicity of the source material from which the gene was obtained, known naturally occurring toxicants in this source material, or various environmental/ecological issues associated with the source material or the gene product. If a gene is selected from a source with a known history of allergy such as peanuts, tree nuts, fish, etc., or even ragweed, birch trees, or grass (common sources of pollen allergens), then assurance must be sought that the gene product is not the allergen from that source. However, such issues may not always be so obvious. For example, chitinase genes might be selected to prevent various fungal diseases common to some crop plants. But chitinases from some sources are allergens so the possible cross-reactivity with other chitinases needs to be assessed (1).

Bt Proteins

Bacillus thuringiensis proteins used to produce various insect-resistant crops serve as a good example of the considerations involved at the gene discovery stage. Genes from *Bacillus thuringiensis* that code for the production of Bt proteins have been cloned into several different agricultural crops to provide insect resistance. Bt proteins are derived naturally from

Bacillus thuringiensis; many different forms of Bt are known to exist (2). Bt in the form of agricultural sprays has been a commercial option for insect control for several decades especially by organic farmers but also in conventional agriculture and by home gardeners. The microbial products used as Bt sprays contain the Bt proteins as the active insecticidal component. The Bt proteins exhibit selective toxicity to specific insect targets but are essentially nontoxic to mammalian species. The Bt proteins present in these commercial insecticides have been subjected to toxicological assessment including acute, subchronic, and chronic toxicity testing in experimental animals and even oral gavage studies in humans (3). Thus, the Bt proteins in these commercial products are widely recognized as safe and effective products. Additional studies conducted with Bt crops produced through agricultural biotechnology confirm their safety as well. Such Bt crops as corn, cotton, and potatoes have been approved by regulatory authorities in the United States and other countries and have been on the market since the mid-1990s.

LINE SELECTION STAGE

After an appropriate and promising gene has been discovered, efforts are undertaken to produce the desired transformation in the host plant and develop the genetically modified crop. During the line selection stage, the genetically modified seed progresses from the laboratory through a variety of greenhouse and field trials. Eventually, if the results are promising, field trials will be conducted. Regulatory approvals usually are needed to proceed to field trials. The safety of the genetically modified crop will be examined on a preliminary basis even at this precommercialization stage. In the United States, after the completion of greenhouse and field trials, the developer must submit a full package of data generated from those trials to the U.S. Department of Agriculture (USDA) to request that the genetically modified crop be moved to nonregulated status. The USDA invites public comment at this point in the approval process. Once USDA moves the crop to nonregulated status, the crop can be grown, tested, or used for traditional crop breeding without further USDA approval.

Agronomic Traits

A number of agronomic traits are considered during the growth trials that occur in laboratories, greenhouses, and fields. These traits might include plant height, leaf orientation, leaf color, early plant vigor, root strength, and of course yield. At this stage, the biological and agronomic equivalence of the genetically modified crop must be compared with its traditional counterpart. Although not specifically done for safety assessment, potential

products with unusual characteristics are eliminated during this stage of development, and the elimination of such products with unusual agronomic or biological characteristics likely enhances the prospects of selecting a safe product. Many prospective novel varieties are discarded from development at this line selection stage.

In the United States, additional governmental scrutiny occurs when the novel plant variety contains pesticidal properties such as Bt proteins. The Environmental Protection Agency (EPA) also is involved in the regulatory approval process for crops with pesticidal properties. In the EPA regulatory process, the developer must first obtain an experimental use permit before planting more than 10 acres of a crop that possesses a pesticidal protein. Public comments are invited at this stage of the review process. Later in the developmental process, the EPA establishes limits (tolerances) on the amount of the pesticidal component that can be permitted in food derived from the genetically modified plant. Then, the EPA reviews data on human, animal, and environmental safety of the pesticidal component. If considerable data already exist on the safety of the pesticidal component and if there is a history of safe use, an exemption from the requirement for a tolerance can be requested, but EPA must rule on whether such an exemption should be granted. Typically, an extensive safety assessment is conducted by EPA at this stage, and public comments are sought on the establishment of a tolerance.

PRODUCT ADVANCEMENT TO COMMERCIALIZATION STAGE

Finally, in the precommercialization stage, the genetically modified product should go through a detailed and more formal safety assessment process. This process should focus on the safety of the gene products associated with the introduced gene and any other likely toxicological or antinutrient factors associated with the source of the novel gene and the crop to which it was introduced. All potential uses of the genetically modified product, both for food and feed uses, must be considered during the safety assessment process. When a prospective genetically modified crops moves from greenhouse and field trials toward commercialization in the United States, the Food & Drug Administration (FDA) gets involved. The FDA has the responsibility for assessing the safety of food produced from the genetically modified crops. During the existing voluntary safety assessment approach, the FDA typically meets with the developer early in the developmental process and offers guidance on the studies that should be performed, in the FDA's opinion, to provide assurance that the genetically modified food will be safe for its intended food and animal feed uses. The nature of the announced, but not yet implemented, mandatory safety assessment approach is not known at this time. The specifics of the safety assessment process likely vary depending upon the nature of the genetically

modified food. The FDA has published a list of questions appropriate in the evaluation of the safety of genetically modified foods and feeds (4). Despite the past voluntary nature of the safety assessment in the United States, all current genetically modified foods in the U.S. marketplace have been subjected to this FDA scrutiny.

Standards

Several worldwide organizations including the Food & Agricultural Organization (FAO) of the United Nations, the World Health Organization (WHO), and the Organization for Economic Cooperation & Development (OECD) have established the background for the safety assessment of foods produced through agricultural biotechnology (5, 6). The general conclusion of these reports is that the products of plant biotechnology are not inherently less safe than those developed by traditional breeding (7). Another critical conclusion is that the food safety considerations are basically of the same nature as those arising from the products of conventional breeding, thus traditional approaches to safety assessment are appropriate in the assessment of genetically modified foods. The accepted standard for genetically modified crops is identical to that expressed in the U.S. food laws for all food products—a reasonable certainty that no harm will result from intended uses under anticipated conditions of consumption. Since genetically modified foods have been subjected to extensive safety assessments, whereas foods produced through conventional breeding have not, the argument could be made that foods produced through agricultural biotechnology provide equal or greater assurance of safety by comparison to foods derived through conventional breeding practices.

Substantial Equivalence

From the outset of deliberations on the safety assessment of genetically modified foods, the concept of substantial equivalence has been a key feature (7, 8). The concept of substantial equivalence stresses that a determination should be made regarding whether the genetically modified food is as safe as its traditional counterpart. In practice, many genetically modified varieties of traditional crops, such as corn and soybeans, are altered very little from their traditional counterparts. In the concept of substantial equivalence, the safety evaluation then focuses on those differences while assuming that the unchanged components are just as safe as the traditional counterparts. With the concept of substantial equivalence, the genetically modified food (or food component) is compared with its traditional counterpart for such attributes as origins of genes, agronomic parameters, composition including key nutrients, antinutritents and allergens, and consumption patterns.

Three outcomes are possible from the comparisons made through the concept of substantial equivalence (6). In the simplest case, the genetically modified food could be judged to be substantially equivalent to its conventional counterpart. In this case, no further safety testing would be required. However, this possibility rarely occurs. In the most common situations, the genetically modified food could be judged to be substantially equivalent to its conventional counterpart except for the introduced traits. In this situation, the safety testing would focus upon the safety of the introduced trait or gene product. Finally, the genetically modified food could be judged to be not substantially equivalent to the conventional food or food component. More extensive safety assessments would be required including more rigorous nutritional and toxicological assessment. While no such products have yet been released into the commercial marketplace, such products likely will be developed in the future. Thus far, limited discussion has occurred on the nature of the safety assessment process that would be desirable in such cases. Certainly, safety assessments would need to be conducted in a flexible manner depending upon the nature of the novel food product.

In the comparisons made as part of the concept of substantial equivalence, extensive compositional analyses are conducted on the genetically modified crop to compare it with its conventional counterpart. Of course, the composition of the conventional counterpart can vary significantly as a result of varietal differences, climatic conditions, and agronomic conditions. The choice of the conventional counterpart for comparative purposes is extremely important. As part of the comparison, the crops and the foods or food components produced therefrom are compared for protein, carbohydrate, fat, fatty acid composition, starch, amino acid composition, fiber, ash, minerals, vitamins, and other factors. Antinutrient levels are also compared if known antinutrients are present in either the source material for the novel gene or the host plant. The same is true for allergens especially if the source material is known to be allergenic. The allergenicity of the host plant is less of a concern because, for example, consumers with soybean allergy likely avoid all soybeans whether genetically modified or not.

Introduced Trait

For the majority of genetically modified products entering the marketplace to date, the safety evaluation focuses on the introduced trait or gene product. The DNA from the inserted gene in the genetically modified product is not of great concern because DNA in the diet, regardless of source, is not considered to be toxicologically significant (9). Instead, the safety assessment appropriately focuses on the novel protein produced from the inserted gene and any components that might be created in the genetically modified food from the action of that protein if it is an enzyme. Initially, the safety assessment of the novel protein typically involves structural comparisons to

known toxins or allergens based upon amino acid sequence similarities. Usually, the digestive fate of the novel protein also is assessed, because if the novel protein is rapidly digested in simulated gastric digestibility tests, then the protein is unlikely to exert untoward responses such as allergenicity. Another safety assessment approach for the novel protein would be to purify the protein and test it for toxicity in an acute oral toxicity screen in mice or another suitable animal model. Evaluation of the purified protein can be tricky, because assurance must be sought that the purified protein is virtually identical to the protein as expressed in the genetically modified plant. Proteins in plants are often glycosylated. Glycosylation and other post-translational modifications can potentially influence the toxicity of a novel protein. Another approach is to assess the novel protein's heat stability. However, the relevance of this attribute to the safety of the protein is debatable. The application of these various approaches should be flexible based upon the known information about the protein and its possible toxicity. But, of course, it must be sufficient to convince worldwide regulatory agencies that the novel protein is not inherently toxic.

The use of antibiotic resistance markers in the development of genetically modified foods also has raised safety concerns. In addition to the principal gene product, genetically modified foods usually contain antibiotic resistance markers, the most common of which is neomycin phosphotransferase II although the safety of the common antibiotic resistance markers has been well established (5, 10). This part of the safety assessment will become more important if novel markers are used.

The assessment of the allergenicity of the novel protein is also important. Since all food allergens are proteins, some possibility exists that any newly introduced protein will become a novel allergen. However, foods contain many thousands of proteins and only a few hundred are known to be allergens. Thus, the likelihood that the novel protein will be an allergen on a probabilistic basis alone is slight.

Decision Tree

The FAO and WHO recently developed a decision-tree strategy to assess the potential allergenicity of the novel proteins from genetically modified foods. This strategy (Figure 1) encompasses the source of the novel gene(s) and the allergenicity of the source material for the novel gene, the degree of amino acid sequence homology of the novel protein to known allergens, the immunoreactivity of the novel protein with serum IgE from humans with known allergies to the source material, the immunoreactivity of the novel protein with serum IgE from humans with known allergies to materials that are broadly related to the source material for the gene (e.g., to grass pollen for genes obtained from monocots), the resistance of the novel protein to pepsin

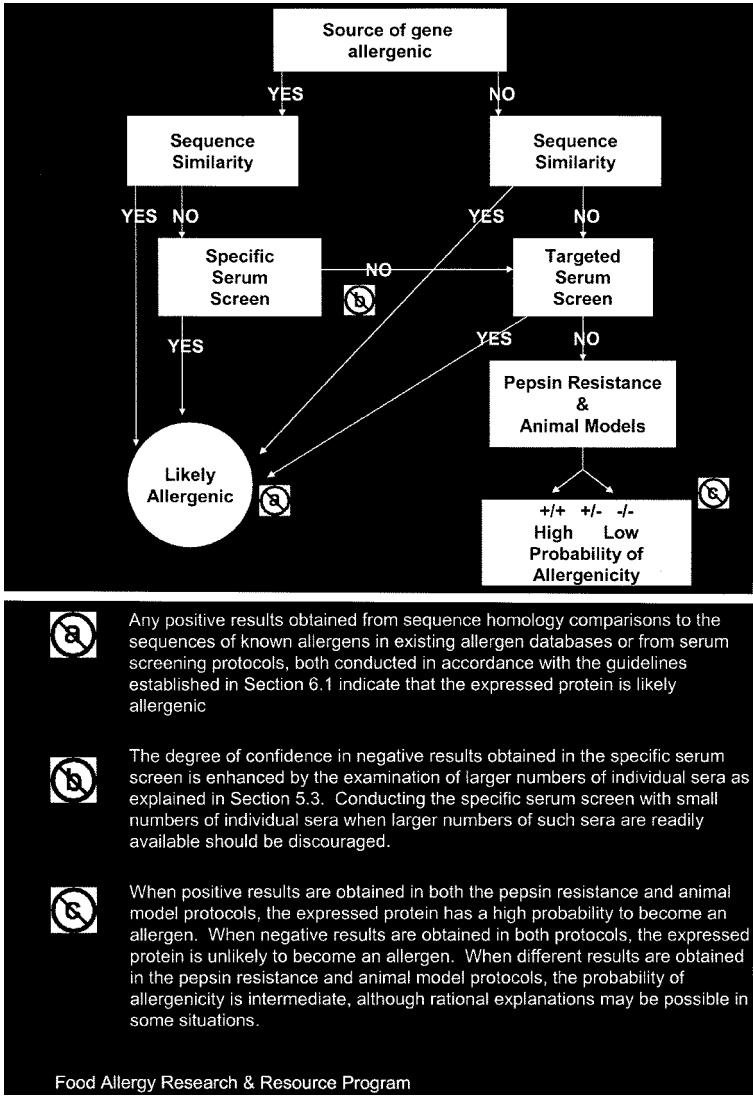


FIGURE 1 FAO/WHO Decision tree strategy for assessment of the potential allergenicity of novel proteins from genetically modified foods.

which would be an indication of its digestive stability, and the immunogenicity of the novel protein in appropriate animal models (11). This new decision-tree strategy is a more conservative approach than a previously developed decision tree, the so-called ILSI/IFBC decision tree (12).

Both decision-tree approaches focused on the source of the gene and the allergenic history of that material. The degree of amino acid sequence homology also is a key strategy in both approaches. However, in the FAO/WHO decision tree the criteria for significant homology has been tightened to 35% overall sequence homology or 6 contiguous identical amino acids, whereas the earlier approach used a criterion of 8 contiguous identical amino acids. Both strategies used immunoreactivity of the novel protein with serum IgE from humans with known allergies to the source material when the gene was obtained from a known allergenic source. However, since not all allergens have been identified, the new decision tree adds targeted serum screening where the immunoreactivity of the novel protein is assessed with serum IgE from humans with known allergies to materials that are broadly related to the source material. Examples would include serum from grass pollen-allergic individuals if the gene is obtained from a monocot source or serum from individuals with cockroach allergy if the gene is obtained from an insect source.

Digestive stability as evaluated by the degree of pepsin resistance is a feature of both decision trees. While the earlier decision tree advocated research to develop animal models to assess the potential allergenicity of the novel proteins, the FAO/WHO decision tree includes use of appropriate animal models as one of the recommended approaches. This feature likely will be controversial since no well-validated animal models exist for such purposes. We hope this recommendation will spur the necessary research to develop such animal models.

A similar approach to these decision-tree strategies was used quite effectively to determine that a high-methionine protein from Brazil nuts introduced into soybeans to correct their inherent methionine deficiency was actually the previously unidentified, major allergen from Brazil nuts (13). As a result, commercialization of the high-methionine soybeans was not pursued.

Safety assessments should also consider possible uses for the genetically modified crop in animal feeding. Corn, soybeans, canola, and cottonseed are among the genetically modified crops important in feeding domestic animals. Feed safety assessment typically involves feeding studies with the appropriate target animal species and comparisons for typical performance parameters such as growth rate.

CONCLUSIONS

In the United States and other countries, genetically modified foods go through numerous regulatory screens between the early gene discovery stage

and product commercialization. As a result, the products of agricultural biotechnology currently sold for food purposes around the world have been subjected to intensive safety evaluations. The safety assessment of genetically modified foods usually begins with a comparison of the novel food with its conventional or traditional counterpart. With the currently marketed genetically modified foods, the novel food is comparable to its conventional counterpart except for a few defined differences resulting from the introduction of the particular gene(s) of interest. In such cases, the safety assessment is then focused upon the safety of the introduced gene and especially the novel gene product or protein.

The currently marketed genetically modified foods are well documented to be safe for their intended uses under the anticipated conditions of consumption. With the future introduction of genetically modified foods that are not comparable to any traditional food, the safety assessment process will become more difficult, although no such genetically modified crops have yet been introduced into the marketplace. A regulatory system exists on a worldwide basis to assure that suitable testing will be conducted to assure the safety of current and future novel foods for consumers before their introduction into the marketplace.

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