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Will genetically modified foods be allergenic?

Steve L. Taylor, PhD, and Susan L. Hefle, PhD *Lincoln, Neb*

Foods produced through agricultural biotechnology, including such staples as corn, soybeans, canola, and potatoes, are already reaching the consumer marketplace. Agricultural biotechnology offers the promise to produce crops with improved agronomic characteristics (eg, insect resistance, herbicide tolerance, disease resistance, and climatic tolerance) and enhanced consumer benefits (eg, better taste and texture, longer shelf life, and more nutritious). Certainly, the products of agricultural biotechnology should be subjected to a careful and complete safety assessment before commercialization. Because the genetic modification ultimately results in the introduction of new proteins into the food plant, the safety, including the potential allergenicity, of the newly introduced proteins must be assessed. Although most allergens are proteins, only a few of the many proteins found in foods are allergenic under the typical circumstances of exposure. The potential allergenicity of the introduced proteins can be evaluated by focusing on the source of the gene, the sequence homology of the newly introduced protein to known allergens, the expression level of the novel protein in the modified crop, the functional classification of the novel protein, the reactivity of the novel protein with IgE from the serum of individuals with known allergies to the source of the transferred genetic material, and various physicochemical properties of the newly introduced protein, such as heat stability and digestive stability. Few products of agricultural biotechnology (and none of the current products) will involve the transfer of genes from known allergenic sources. Applying such criteria provides reasonable assurance that the newly introduced protein has limited capability to become an allergen. (*J Allergy Clin Immunol* 2001;107:765-71.)

Key words: *Biotechnology, allergy, food, sequence homology, digestion, exposure, classification, heat stability*

FOODS PRODUCED THROUGH AGRICULTURAL BIOTECHNOLOGY

Agricultural biotechnology will have tremendous implications for all of agriculture and for the consuming public

Abbreviations used

FAO: Food and Agriculture Organization of the United Nations
IFBC: International Food Biotechnology Council
ILSI: International Life Sciences Institute
WHO: World Health Organization

in the coming years. From the perspective of production agriculture, agricultural biotechnology will likely be a major revolution in agriculture worldwide. From a consumer perspective, agricultural biotechnology holds great promise for foods with improved quality characteristics, improved nutritional and health attributes, resistance to spoilage, and even reduced levels of allergens.¹

With the growing worldwide population, the pressure on agriculture to provide sufficient food to feed the masses will continue to escalate. Conventional modern farming practices will simply not suffice with the current constraints on arable land. Yet much of the potential harvest is lost each year to insect pests, competition from weeds, drought conditions, and other agronomic factors. Although the application of conventional agricultural chemicals can eliminate insects and weeds, many consumers are concerned about the safety of pesticide residues in their foods and the potential adverse environmental effects associated with these practices. Agricultural biotechnology already offers crops with improved insect resistance and herbicide tolerance that allow the control of losses caused by insects and weeds with limited agrichemical application. Improved tolerance to climatic stresses and poor soil conditions will likely be developed through agricultural biotechnology in the foreseeable future, and this improved technology is contained in the seed itself, so that the benefits can be enjoyed by farmers who do not have access to modern and expensive agricultural machinery.

The products of agricultural biotechnology are already appearing in American and worldwide diets. Planting of insect-resistant corn and herbicide-tolerant soybeans began in the mid-1990s and has now reached 30% to 50% of the total acreage planted for these crops in North America. As a result, food products containing ingredients derived from such corn and soybeans are quite common in the consumer marketplace.

From the University of Nebraska, Food Allergy Research and Resource Program, Lincoln.

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Reprint requests: Steve Taylor, PhD, 143 Food Industry Complex, Lincoln, NE 68583-0919.

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Consumers may perceive few benefits from agricultural biotechnology because the major existing benefits are improved agronomic traits, such as insect resistance or herbicide tolerance. A few foods from agricultural biotechnology with direct consumer benefits have reached the marketplace, including tomatoes with improved ripening characteristics that provide enhanced flavor attributes.² These products are not yet widely available. Canola oil enriched with high levels of oleic acid, a monounsaturated fatty acid that is thought to be beneficial to cardiovascular health, may soon be available.³ Much publicity has surrounded the development of the so-called golden rice with enhanced levels of vitamin A.⁴ Although golden rice is not yet available commercially, it holds some promise to help eradicate widespread vitamin A deficiency and night blindness in certain Asian populations.⁵ Agricultural biotechnology offers the promise of many more such advances, including the reduction or elimination of allergenic proteins from commonly allergenic foods.

Only a small number of crops have thus far been commercially developed through agricultural biotechnology,⁶ including corn, potatoes, canola, soybeans, and cotton, with either improved insect resistance or enhanced herbicide tolerance. Virus-resistant squash and papaya have been introduced into the market. The Hawaiian papaya industry that was virtually destroyed by a viral infection has been revitalized through this development.

Many more traits can potentially be developed in these and other crops in the near future. Within a few years, the potential exists for dozens of new products developed through agricultural biotechnology to enter the marketplace, including crops that protect themselves from diseases and pests; crops that prosper under adverse conditions, such as heat, cold, and drought; and crops that look better, taste better, and provide better nutrition.

SAFETY OF FOODS PRODUCED THROUGH AGRICULTURAL BIOTECHNOLOGY

Governmental regulatory agencies in most developed countries require a mandatory safety assessment and consultation with government regulators before allowing commercial sale. Although the safety assessment approach in the United States has been voluntary rather than mandatory, the Food and Drug Administration has pledged to propose and develop a mandatory system in the near future.

The crops that are currently on the market have been thoroughly assessed for their safety under approaches recommended by the World Health Organization (WHO), the Food and Agriculture Organization of the United Nations (FAO),^{3,7} and other worldwide organizations.

The alterations introduced into most such crops are minor and highly targeted, involving the insertion of one or a few genes and their gene products into the new variety. Obviously, the remainder of the plant genome would be predicted to remain exactly the same as it had been previously. Thus the safety assessment of crops produced through agricultural biotechnology has often focused on

the concept of substantial equivalence. Corn or soybeans with one or a few new introduced traits remain substantially equivalent to their traditional counterparts. Thus the safety assessment is focused on those genes and their products that are introduced into the novel variety. Because ingested DNA is considered highly digestible and safe regardless of its source,⁸ the safety assessment process typically is focused on the proteins expressed from the introduced novel genes. Also, if the host or donor plants are known to contain any antinutritional, toxic, or allergenic components, the novel plant would be tested to determine whether the concentration of those components had been altered during the process of creating the new variety. If the novel plant is considered not to be substantially equivalent to its traditional counterpart because a larger number of genes has been introduced or the nutritional content has been altered in some significant manner, then a more thorough safety evaluation would be necessary. However, no such crops have yet been commercially developed, and therefore the exact nature of the safety assessment that will be required by various government regulatory agencies around the world is not yet known.

Among the key issues involved in the safety assessment of foods produced through agricultural biotechnology is an assessment of the potential allergenicity of the novel proteins introduced into these foods. The remainder of this review will be focused on the assessment of the allergenicity of these new food products.

ASSESSMENT OF THE ALLERGENICITY OF FOODS PRODUCED THROUGH AGRICULTURAL BIOTECHNOLOGY

Because the crops produced through agricultural biotechnology ultimately result in the introduction of new proteins, the potential allergenicity of the newly introduced proteins must be assessed. Only a few of the many proteins found in foods are allergenic under the typical circumstances of exposure. However, because virtually all allergens are proteins, the allergenic potential of the novel proteins must be determined in every case. In 1996, a task force of the International Food Biotechnology Council (IFBC) and the Allergy and Immunology Institute of the International Life Sciences Institute (ILSI) developed a decision tree approach for the assessment of the potential allergenicity of plants produced through agricultural biotechnology.⁹ This approach focused on evaluating the source of the gene, the sequence homology of the newly introduced protein to known allergens, the immunoreactivity of the novel protein with serum IgE from individuals with known allergies to the source of the transferred genetic material, and various physicochemical properties of the newly introduced protein, such as heat stability and digestive stability. The decision tree strategy, as developed by IFBC/ILSI⁹ and modified by the FAO/WHO,³ is depicted in Fig 1. Although the application of such criteria provides reasonable assurance that the newly introduced

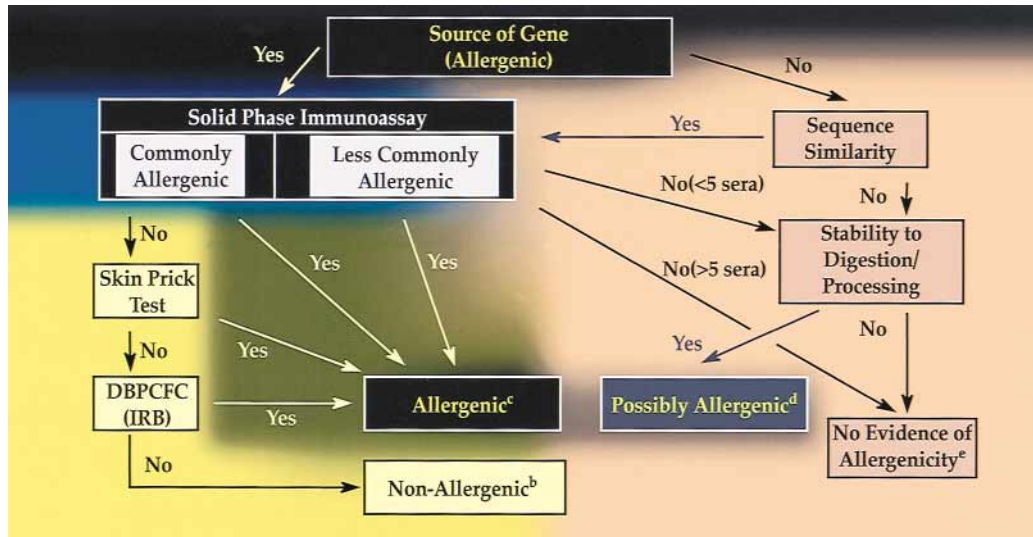


FIG 1. Assessment of the allergenic potential of foods derived from genetically modified crop plants. ^bThe combination of tests involving allergic human subjects or blood serum from such subjects would provide a high level of confidence that no major allergens were transferred. The only remaining uncertainty would be the likelihood of a minor allergen affecting a small percentage of the population allergic to the source material. ^cAny positive results obtained in tests involving allergic 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 labeled to protect allergic consumers. ^dA 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 ($n < 5$) 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. ^eA 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 ($n = >5$ but <14) provides no evidence of allergenicity. Stability testing may be included in these cases. However, the level of confidence based on only 2 decision criteria is modest. It is suggested that other criteria should also be considered, such as the level of expression of the novel protein. *DBPCFC*, Double-blind, placebo-controlled food challenge. (Adapted from the decision tree approach developed by the IFBC and the Allergy and Immunology Institute of the ILSI [Metcalf DD, et al. *Crit Rev Food Sci Nutr* 1996;36(Suppl):S165-86] by FAO/WHO 2000).

protein had limited capability to become an allergen, this decision tree approach has been subjected to some criticism.¹⁰ Accordingly, in January 2001, the FAO and WHO will convene an expert consultation to discuss the development of an improved approach to the assessment of the allergenicity of foods produced through agricultural biotechnology. Additional criteria that will be considered for inclusion into the assessment strategy include the level of expression of the novel protein in the edible portion of the new plant variety, the functional characterization of the novel protein, and the use of animal models to predict allergenicity.

Source of the novel gene

As noted in Fig 1, the sources of genetic material to be introduced can be classified as commonly allergenic, less commonly allergenic, or of unknown allergenic potential. Commonly allergenic foods include peanuts, soybeans, tree nuts, and wheat from the plant kingdom and milk, eggs, fish, and crustacea from the animal kingdom. These few foods are thought to account for more than 90% of all food allergies on

a worldwide basis. These 8 foods or food groups are widely considered to comprise the commonly allergenic foods.¹¹

Many additional foods could be listed as less commonly allergenic. The IFBC/ILSI report contains an extensive list of more than 160 foods and food-related substances that have been associated with allergic reactions in individuals on at least some occasions.¹² Many of these foods should probably be viewed as less commonly allergenic. However, the association of some of these foods with allergic reactions has not been particularly well investigated, and therefore the strength of the clinical information for each source should be considered independently.¹³

If a gene is obtained from a known allergenic source, either commonly allergenic or less commonly allergenic, then a careful assessment must be conducted to ensure that the gene of interest does not encode an allergen. This assessment is particularly important when the novel protein is expressed in the edible portion of the new plant variety and assumes much less importance if it is only expressed in portions of the plant that are not eaten.¹³ The assumption must be made that the source gene in

such circumstances encodes for an allergen unless data are generated to disprove that assumption. Obviously, greatest concerns are raised when the gene is obtained from a commonly allergenic source.

In many cases in agricultural biotechnology, the gene is obtained from a source with no history of allergenicity. Genes derived from bacteria, viruses, weeds, and ornamental plants would be examples. The products of these genes would be classified as proteins with an unknown allergenic potential.

Sequence homology to known allergens

A comparison of the amino acid sequence homology of the novel protein to the amino acid sequences of known allergens is a useful initial approach in the determination of allergenic potential.⁹ The amino acid sequences of many major allergens, including food allergens, are known. The current criteria used to determine significant sequence similarity, as proposed in the IFBC/ILSI strategy, is a match of at least 8 contiguous, identical amino acids.⁹ The use of 8 contiguous, identical amino acids as a match was predicated on the minimum peptide length for a T cell-binding epitope.⁹ Because this approach assesses the entire protein sequence, it is not based on the identity of amino acid sequences just to known T cell- and B cell-binding epitopes of known allergens. Thus this approach may identify matching sequences that are unrelated to the allergenic potential of the novel proteins.

However, although use of amino acid sequence homology has been widely supported, the choice of 8 contiguous, identical amino acids has been criticized. The exact conservation of epitope sequences may not be required to allow IgE binding. Thus limited and conservative substitutions may still allow immunoreactivity. Furthermore, the criterion cannot identify discontinuous or conformational epitopes that depend on the tertiary structure of the protein. Although the heat stability of food allergens argues for the importance of linear, continuous epitopes,¹⁴ the importance of discontinuous epitopes has been recognized in a few food allergens, including Ara h 1.¹⁵ Suggestions have been made that the criterion should be reduced from 8 contiguous, identical amino acids to a smaller number, but there is no agreement yet on the ideal number.

Immunoreactivity with serum IgE

If the gene is derived from a known allergenic source or if the search for sequence homology identifies a match with a known allergenic source, then an assessment of the immunoreactivity of the novel protein with IgE antibodies from the sera of individuals allergic to the source material must be conducted.⁹ RASTs or similar tests for IgE binding could be used to determine whether IgE from patient sera react with the novel protein or extracts of the novel food. The availability of sera from well-characterized patients is an important issue and could be rather challenging in cases where the gene was obtained from a less commonly allergenic source. A positive RAST or similar test result would certainly raise concerns about the possible allergenicity of the novel pro-

tein. Unless these concerns were convincingly discounted by additional *in vivo* testing, foods containing these new genes should be considered allergenic.

In the case of negative or equivocal results in the *in vitro* immunoassays, the novel food should be investigated further by using *in vivo* skin prick tests¹⁶ and ultimately confirmed with double-blind, placebo-controlled food challenges with allergic individuals.¹⁷ A positive result in either of these *in vivo* tests would be sufficient evidence of the allergenicity of the novel food.

This approach has already proven useful in the assessment of the potential allergenicity of a novel variety of soybeans. Pioneer Hi-Bred International, now a division of DuPont, developed a high-methionine variety of soybeans by cloning a high-methionine protein from Brazil nuts into soybeans. Soybeans are inherently deficient in methionine, and therefore farmers must supplement animal diets with methionine when feeding soybean meal to farm animals. The high-methionine protein from Brazil nuts represented a significant fraction of the total protein in the novel variety of soybeans. At the time of this development, the allergenicity of Brazil nuts was well established,¹⁸ but the identity of the allergens in Brazil nuts was not known. Nordlee et al¹⁹ evaluated the possible allergenicity of these novel soybeans and the purified high-methionine protein by using blood sera from individuals with documented Brazil nut allergy. RAST inhibition results showed that the gene obtained from the Brazil nut likely encoded for an allergen (Ber e 1), and this possibility was confirmed by positive skin prick test responses in 3 of the individuals with Brazil nut allergy.¹⁹ As a result, Pioneer Hi-Bred International decided not to commercialize this variety of soybeans.

In the more common situation, genes are obtained from sources with no history of allergenic potential, and then immunoreactivity cannot be used as a criterion. In these cases other criteria must be used for the allergenicity assessment. In the IFBC/ILSI approach, sequence homology and physicochemical stability were the 2 criteria applied to novel proteins produced from such genes (Fig 1).

For sources with no allergenic history, there have been suggestions for screening of the transgenic protein with serum from atopic individuals for use in predicting the potential for allergenicity. There are several reasons why this would not be a useful approach. The predictive accuracy for *in vitro* methods for food allergens can vary from only 50% to more than 90%.^{20,21} The presence of clinically insignificant IgE^{22,23} clouds the dependability of these types of assays for *in vivo* extrapolation and can be very pronounced for some plant proteins.²⁴ Also, *in vitro* IgE binding can give an indication that the substance in question has the ability to bind IgE but does not prove that this binding would occur *in vivo*, with consequent degranulation of mast cells and basophils. In addition, high variability exists between atopic individuals in terms of levels of total and specific IgE. Use of commercial serum banks by companies attempting to meet screening requirements would result in poorly characterized or self-diagnosed serum samples to be used in such

testing, creating questions about the validity of the results. Hence there is little or no value in screening atopic sera for prediction of allergenicity of products produced through agricultural products.

Physicochemical stability of the novel protein

The digestive stability of the novel protein is a useful criterion in the assessment of the protein's allergenic potential. To become allergenic, a protein must reach the intestinal tract in a form that is sufficiently intact to provoke the immune system. If the protein is rapidly digested under simulated gastric and intestinal digestive models, that prospect seems unlikely. In simulated gastric and intestinal digestive models, known food allergens exhibited greater proteolytic stability than known nonallergenic food proteins.²⁵ Many of the novel proteins introduced into foods produced through agricultural biotechnology were also rapidly digested in these same model systems.²⁵ For example, the enzyme transferred into soybeans to make them tolerant to the herbicide glyphosate is rapidly digested *in vitro*²⁶ and is therefore unlikely to induce allergic sensitization.

Although the applicability of the criterion of digestive stability is widely accepted, a need exists to develop a standard protocol for the simulated gastric and intestinal model systems. Obviously, no model system will mimic humans with limited gastric acid secretion and other gastrointestinal maladies,¹³ but important comparative data can be obtained by using a standardized model system.

Level of expression of the novel protein

In many cases the novel proteins are expressed at very low levels in foods produced through agricultural biotechnology. Certainly in the cases of insect resistance and herbicide tolerance, the current products on the market contain rather low levels of the novel proteins, but those levels are sufficient to provide the modified crop with the enhanced agronomic benefits.

Metcalfe et al⁹ suggested that the level of expression was probably an important factor to consider in assessing the allergenicity of foods produced through agricultural biotechnology, but the level of expression of the novel protein was not actually included in the decision tree strategy, as outlined in Fig 1.

Emerging evidence suggests that a threshold dose does exist below which allergic individuals will not react adversely to the offending food. Experience with double-blind, placebo-controlled trials suggests that the threshold dose for peanut protein is in the neighborhood of 1 to 20 mg.²⁷⁻³⁰ Apparently, if an allergenic protein were expressed in a food produced through agricultural biotechnology at levels well below 1 mg per serving, the hazard for allergic consumers would be minimal.

Because the genes transferred through agricultural biotechnology are often obtained from sources with no history of allergenicity, the threshold dose for sensitization to a novel protein is also an important consideration. Certainly, because most proteins are not allergenic, one

should not assume that all of these proteins possess that potential, but very little information exists on the threshold dose for sensitization. The best information may come from studies on exclusively breast-fed infants because some exclusively breast-fed infants experience allergic reactions on their first documented exposure to certain allergenic foods.³¹⁻³³ Of course, these infants may have had surreptitious or adventitious exposures to these allergenic foods, and therefore these observations must be viewed cautiously. However, the possibility exists that these infants are sensitized to certain food allergens that are ingested by the mother and expressed at rather low levels in the breast milk. Data on secretion of bovine β -lactoglobulin and egg ovalbumin in breast milk indicate that wide variability exists in the levels of such allergens in breast milk.³⁴⁻³⁶ However, an analysis of this information would suggest that these infants would be exposed to levels of 30 to 230,000 μ g of β -lactoglobulin and 60 to 1750 μ g of ovalbumin over the period of 1 year of exclusive breast-feeding.

International discussion and debate is needed to determine how best to use the level of expression of the novel protein in the assessment of the allergenicity of foods produced through agricultural biotechnology.

Functional characteristics of the novel protein

Many common plant allergens fall within a few functional categories. The pathogenesis-related proteins of several different types are prominently involved.³⁷ If the novel proteins introduced into foods developed through agricultural biotechnology fall into functional categories that contain known food allergens, great caution must be exercised in assessing the potential allergenicity of these particular proteins. This criterion merits some discussion as an additional criterion in the allergenicity assessment strategy.

Animal models for predicting allergenicity

Well-validated animal models do not exist for prediction of the allergenicity of novel proteins. Several animal models, especially the Brown Norway rat, appear to be promising.³⁸⁻⁴⁰ However, further research is needed on the development of animal models to assess their predictive accuracy. If a well-validated, predictive animal model could be developed, it should definitely be included in the allergenicity assessment strategy.

LABELING OF FOODS PRODUCED THROUGH AGRICULTURAL BIOTECHNOLOGY

The US Food and Drug Administration has clearly indicated that if any food produced through agricultural biotechnology presented any specific concerns regarding allergenicity, such foods should be labeled. The exact nature of the labeling has not been determined because there are no existing examples in which such labeling would be required. However, this labeling would need to provide the necessary and essential information to potentially affected allergic consumers. Most other countries

have adopted an attitude similar to that of the United States regarding the mandatory labeling of foods produced through agricultural biotechnology that raise specific allergenicity concerns.

Some consumer groups opposed to agricultural biotechnology have repeatedly called for mandatory labeling of foods produced through agricultural biotechnology. Such labeling would be generic and would thus be of limited value to consumers with specific food allergies. A thorough review of the merits and demerits of mandatory labeling has recently been published.⁴¹

THE STARLINK SITUATION AS AN EXAMPLE

Recently, considerable publicity has surrounded one particular variety of corn, known as Starlink, and its potential to be allergenic. Starlink, an insect-resistant corn variety, contains a novel gene from a naturally occurring soil bacterium, *Bacillus thuringiensis*, that encodes for a form of the Bt toxin known as cry9c protein. Several hundred different types of Bt toxins are known to occur naturally with differing insecticidal properties.⁴² Bt toxins are toxic to certain insect species but generally harmless to humans.⁴³ Other forms of Bt toxin have been introduced into other varieties of corn, such as the cry1a(b) gene in Monsanto's YieldGuard corn, and Bt sprays have been widely used in agriculture for decades.

Starlink corn and the cry9c protein were subjected to safety-allergenicity assessment by Aventis Corporation. The gene came from a source with no history of allergenicity. The cry9c protein was not structurally homologous to any known food or environmental allergens, but the cry9c protein was more resistant to digestion than other forms of the Bt toxin. Because resistance to digestion is an attribute shared with some food allergens²⁵ and because digestive stability was a key criterion in the IFBC/ILSI decision tree strategy (Fig 1), the US regulatory agencies were concerned about the potential for this protein to become a new food allergen. Although digestive stability alone is not fully predictive of potential allergenicity, this finding caused the US regulatory authorities to delay approval of Starlink corn for human food consumption.

However, Starlink corn was approved by US regulators for animal feed, and small numbers of acres were planted in the 1998, 1999, and 2000 growing seasons. The furor developed when it was discovered that the segregation of Starlink corn for animal feeding had been imperfect. Because Starlink corn was not approved for human food, any foods found to contain evidence of Starlink were recalled from the marketplace by the manufacturers. Unfortunately, the news media often portrayed Starlink as an allergen or a potential allergen, causing consumer concerns.

In fact, the cry9c protein is probably not a food allergen under the current circumstances of exposure. Consumer exposure to cry9c protein through corn-based food products has been extremely slight because only 0.05% of all harvested corn was Starlink, most of this corn was likely diverted to animal feed, the cry9c protein is

expressed at only 0.0129% in the corn kernel, and many corn-based food products contain little corn protein. Thus consumer exposure to cry9c protein currently has likely been insufficient to elicit allergic sensitization. Because commercialization of Starlink has ceased, it is unlikely that the cry9c protein will become a food allergen.

This episode points out the importance of assessing the allergenicity of foods produced through agricultural biotechnology. In fact, the assessment process prevented the approval of Starlink corn for human consumption. Its approval as animal feed was questionable but likely posed little risk to allergic consumers.

CONCLUSIONS

Foods produced through agricultural biotechnology should be assessed for their potential allergenicity. Although strategies exist for such assessments, improvements should be considered, especially in cases in which the gene of interest is derived from a source with no history of allergenicity. However, widespread agreement by regulatory authorities around the world on the nature and application of various criteria in the assessment of the allergenicity of foods produced through agricultural biotechnology is needed.

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