

Safety Assessment of Genetically Modified Foods¹

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Abstract: The development of novel foods produced through agricultural biotechnology is a complex three-stage process: gene discovery, line selection, and product advancement to commercialization. The safety of genetically modified foods is an integral part of the overall developmental process throughout all of the stages. In the discovery stage, the safety of the gene, its source, and the gene products must be considered. If any questions arise at this stage, these questions must be answered later in the developmental process. During the line selection stage, the genetically modified seed progresses through a variety of greenhouse and field trials. At this stage, the biological and agronomic equivalence of the genetically modified crop to its traditional counterpart must be compared. While the evaluations made during this stage are not specifically directed toward a safety assessment, many potential products with unusual characteristics are eliminated during this stage of development. However, the elimination of products with unusual agronomic or biological characteristics enhances the likelihood that a safe product will be generated. Finally, in the pre-commercialization stage, the genetically modified product undergoes a detailed safety assessment process. This process focuses on the safety of the gene products associated with the introduced gene and any other likely toxicological or anti-nutrient factors associated with the source of the novel gene and the crop to which it was introduced. The safety of the genetically modified product for both food and feed uses is considered. Thus far, all of 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 world. The current generation of genetically modified products are quite safe for human and feed animal consumption.

Key words: allergen, antibiotic resistance, food safety, genetic engineering, safety assessment, substantial equivalence, toxicant.

The introduction of foods produced through agricultural biotechnology has generated considerable concern among some consumers in many countries. The safety of these novel products has often been called into question during the ongoing public debate. The assertion has often been made by opponents of this technology that foods produced through agricultural biotechnology have not been assessed for safety before their release to the market. In fact, that assertion is untrue. The foods produced through agricultural biotechnology have been thoroughly assessed for their safety before their release to the commercial market. The safety assessment data have been reviewed by regulatory agencies around the world and have been found to be satisfactory to allow the products of these crops to enter the food system in the majority of cases. The sole exception in the United States has been Starlink™ corn containing the cry9c gene that had been approved only for use in animal feed due to some uncertainty about the potential of the Bt protein emanating from that gene to become an allergen if allowed in foods. Some confusion regarding the extent of the safety assessment has arisen because, in the United States, the safety assessment has been voluntary. However, in May 2000, the U.S. Food and Drug Administration announced its intent to create a required approach to safety assessment of genetically modified foods. Hopefully, the development of a required safety assessment process for genetically modified foods will inspire more consumer confidence even though there are no known safety concerns regarding any of the products of agricultural bio-

technology that are currently allowed in the consumer food marketplace.

The development of novel foods through agricultural biotechnology involves a complex process that can be viewed as occurring in three stages: gene discovery, line selection, and product advancement to commercialization. The safety assessment process for each new crop should be an integral part of each of the phases of this overall process.

Gene discovery stage: In the gene discovery stage, the scientist develops a product concept and then screens and selects genes that might allow the fulfillment of that concept. Safety assessment must begin at the gene selection phase. The selection of suitable genes should take into account their source and previous consumer exposure to this source material, the history of safe use of the source material, the gene, the product of the gene, and any ethical issues that might occur. Environmental and ecological concerns also should be considered at this early stage, but that subject is beyond the scope of this particular review. The National Institutes of Health (NIH) have established guidelines for product developers to follow after a scientist discovers a potentially marketable product concept. Under NIH guidelines, the developers are urged to assemble a Biosafety Committee made up of employees of the company developing the product and members of the general public. The Biosafety Committee reviews the environmental and health risks that might be posed by the development of the proposed product. If unacceptable risks are identified at this stage, the Biosafety Committee would recommend that the concept not be developed.

Obviously, this initial safety assessment at the gene discovery phase is extraordinarily important because it can draw attention to concerns and highlight questions that must be effectively addressed later in the safety assessment process if the concept is not abandoned at

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this point. Examples of concerns that might be raised at this point include the allergenicity of the source of the gene, known naturally occurring toxicants in the source of the gene, or environmental/ecological issues associated with the source of the gene or the gene product. If the developer selects a gene from a source with a known history of eliciting allergenic responses such as peanuts, tree nuts, fish, etc., then assurance must be sought that the gene product is not the allergen from that source. However, such issues are often not directly obvious. For example, chitinase genes might be selected as a means to prevent various fungal diseases common to some crop plants. But, chitinases from some sources are allergens (Breitenender and Ebner, 2000) so the possible cross-reactivity with other chitinases needs to be assessed.

The situation with the Bt proteins used to produce various insect-resistant crops serves as a good illustration of the considerations involved at the gene discovery stage. Bt proteins are derived naturally from *Bacillus thuringiensis*; many different forms of Bt are known to exist (Schnepf et al., 1998). Bt has been a commercial option for insect control in the form of sprays for several decades. Bt sprays have been used quite commonly by organic farmers and have been widely used 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 present in these commercial insecticides have been subjected to toxicological assessment including acute, subchronic, and chronic toxicity testing in experimental animals and oral gavage studies in humans (McClintock et al., 1995). The Bt proteins in these commercial products, widely recognized as being safe and effective, exhibit selective toxicity to specific insect targets but are essentially nontoxic to mammalian species. Studies conducted with Bt crops produced through agricultural biotechnology confirm their safety as well. Various Bt crops, including corn 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: At the conclusion of the gene discovery phase, laboratory experiments are conducted to achieve the desired transformation and produce the genetically modified crop. At this stage, several regulatory hurdles must be passed in the United States. First, the USDA must review and approve plans for greenhouses and other facilities where the plants will be developed and tested. Second, the developer must seek and obtain USDA approval to conduct field trials. Third, the USDA must give approval to the developer to allow shipment of seeds from the greenhouse to the field trial site. After the completion of greenhouse and field trials, the developer must submit a full package of data generated from those trials to the USDA to request that the genetically modified crop be moved to non-

regulated status. The USDA invites public comment at this point in the approval process. Once USDA approves the crop at this stage, the crop can be grown, tested, or used for traditional breeding without further USDA action.

During laboratory, greenhouse, and field trials, a number of agronomic traits are taken into consideration. These traits include such attributes as plant height, leaf orientation, leaf color, early plant vigor, root strength, and yield. While these agronomic traits are not specifically linked to any safety assessment, this agronomic evaluation leads to the elimination for development of any varieties that have unusual agronomic characteristics. It might be argued that varieties with unusual traits would be more likely than others to present safety concerns if they survived the developmental process to the formal safety assessment stage. Thus, many prospective novel varieties are discarded from development at the line selection stage.

Additional governmental scrutiny occurs in the United States if the novel plant variety contains pesticidal properties such as Bt proteins. In such cases, the Environmental Protection Agency (EPA) also must approve the crop at several stages of the developmental process. First, the developer must obtain an experimental use permit from EPA 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. As the product progresses through development, the EPA must establish limits (tolerances) on the amount of the pesticidal component that can be permitted in food derived from the genetically modified plant. At this step, the EPA reviews data on human, animal, and environmental safety of the pesticidal component. If considerable data should already exist on the safety of the pesticidal component and if there is a history of safe use, the developer can request an exemption from the requirement for a tolerance, but EPA must rule on whether such an exemption should be granted. Usually, an extensive safety assessment is conducted by EPA at this stage. Public comments are also sought on the establishment of a tolerance. However, it should be emphasized that EPA participates in the safety assessment only when pesticidal components are involved in the genetically modified crop. Finally, the EPA conducts a formal review of all data related to genetically modified crops containing pesticidal components and decides whether the crop can be registered for commercial use. Public comments are again invited at this final stage of the EPA process.

Product advancement to commercialization stage: As a prospective genetically modified crop moves from greenhouse and field trials toward commercialization in the United States, the Food and Drug Administration (FDA) has responsibility for assessing the safety of food produced from that crop. Typically, the FDA meets with the developer early in the developmental process and

offers guidance as to what studies FDA considers appropriate to provide assurance that the genetically modified food will be safe for its intended food and animal feed uses. While the process is officially voluntary at present, all current genetically modified foods in the U.S. marketplace have been subjected to this FDA scrutiny. Additionally, the FDA has announced that this process will soon be made mandatory. FDA has published a list of questions that it considers appropriate in the evaluation of the safety of genetically modified foods and feeds (Anonymous, 1992). The specifics of the safety assessment process are likely to vary depending on the nature of the genetically modified food.

Many worldwide organizations, including the Food and Agricultural Organization of the United Nations (FAO), the World Health Organization (WHO), and the Organization for Economic Cooperation and Development (OECD), have established the background for the safety assessment of foods produced through agricultural biotechnology (Anonymous, 2000a, 2000b). The general conclusion of these groups is that the products of plant biotechnology are not inherently less safe than those developed by traditional breeding (Anonymous, 2000c). Furthermore, the food safety considerations are basically of the same nature as those arising from the products of conventional breeding so traditional approaches to safety assessment are appropriate. 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. In reality, foods produced over the years by conventional breeding have not been subjected to extensive safety assessment. By comparison, genetically modified foods have been subjected to extensive safety assessments. Thus, the argument could be made that foods produced through agricultural biotechnology provide equal or greater assurance of safety than foods derived through conventional breeding practices.

From the early 1990s, a key underpinning for the safety assessment process has been the concept of substantial equivalence (Anonymous, 1996, 2000c). The basis of the concept of substantial equivalence is to determine that the genetically modified food is as safe as its traditional counterpart. Obviously, many genetically modified varieties of traditional crops, such as corn and soybeans, will be altered very little from their traditional counterparts. In that sense, the safety evaluation can focus on those small 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 to its traditional counterpart for such attributes as origins of genes, agronomic parameters, composition including key nutrients, anti-nutrients and allergens, and consumption patterns.

Three outcomes are possible from the substantial equivalence comparisons (Anonymous, 1996). First, 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 outcome would occur rather rarely. Second, 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 on the safety of the introduced trait or gene product. This possibility is, by far, the most common outcome of substantial equivalence comparisons at present. 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 for such products, including a more rigorous nutritional and toxicological assessment. However, no such products have yet been released into the commercial marketplace so limited discussion is possible on the nature of the safety assessment process in such cases. Certainly, the safety assessments would need to be conducted in a flexible manner depending on the nature of the novel food product.

In the process of substantial equivalence comparison, extensive compositional analyses are conducted on the genetically modified crop to compare it to the conventional counterpart. Of course, it must be remembered that the composition of the conventional counterpart can vary significantly as a result of dozens of commercial varieties grown under countless types of climatic and agronomic conditions. So, the choice of the conventional counterpart for comparative purposes can be a challenging, but extremely important exercise. 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. If known anti-nutrients are present in either the source material for the novel gene or the host plant, the levels of the anti-nutrients are also compared. The same is true for allergens, especially if the source material is known to be allergenic. The allergenicity of the host plant may be less of a concern because, for example, consumers with soybean allergy will likely avoid all soybeans whether genetically modified or not.

In the majority of cases thus far, the safety evaluation focuses on the introduced trait or gene product. The safety of the DNA from the inserted gene in the genetically modified product is not an issue of great concern because DNA in the diet, regardless of source, is not considered to be toxicologically significant (Beever and Kemp, 2000). The safety assessment typically focuses on the novel gene product or protein and any components that might be created in the genetically modified food from the action of that protein if it is an enzyme. The first step in the safety assessment of the novel protein

often involves structural comparisons to known toxins or allergens based on amino acid sequence similarities. The digestive fate of the novel protein is usually assessed. If the novel protein is rapidly digested in simulated gastric digestibility tests, then the protein is unlikely to exert untoward responses such as allergenicity. The novel protein is typically purified and tested for toxicity in an acute oral toxicity screen in mice or another suitable animal model. When purifying the protein, assurance must be sought that the purified protein is virtually identical to the protein expressed in the genetically modified plant. In plants, proteins are often glycosylated. Glycosylation and other post-translational modifications can potentially influence the toxicity of the novel protein. The heat stability of the novel protein is also determined in many cases although the relevance of this attribute to the safety of the protein is debatable.

In addition to the principal gene product, genetically modified foods usually contain antibiotic resistance markers that remain from the developmental process. The most common marker is neomycin phosphotransferase II. The safety of the common antibiotic resistance markers has been well established (Anonymous, 1993, 2000a), but this part of the safety assessment process becomes more important if novel markers are used.

The assessment of the allergenicity of the novel gene product is another important element of the safety evaluation process. Since all food allergens are proteins, the possibility exists that a newly introduced protein will become a novel allergen. However, of the many thousands of proteins that currently exist in the diet, only a few hundred are known to be allergens so the potential for allergenicity is slight. However, a scheme has been developed to assess the allergenicity of genetically modified foods that encompasses the source of the novel gene(s), the allergenicity of the source of the novel gene, the immunoreactivity of the novel gene product with serum IgE from humans with known allergies to the source material, the sequence similarity of the novel protein to known allergens, and the digestive fate of the novel protein in simulated gastric digestion models (Metcalf et al., 1996). This approach 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 (Nordlee et al., 1996). As a result, commercialization of the high-methionine soybeans was discontinued.

In addition to food safety assessment, many genetically modified crops must be subjected to feed safety assessments. Corn, soybeans, canola, and cottonseed are examples of crops that are important in feeding of domestic animals. The approach to feed safety assessment typically involves feeding studies with the appro-

priate target animal species and comparisons for typical performance parameters such as growth rate.

Conclusions: In the United States, genetically modified foods go through numerous regulatory screens between the early gene discovery stage and product commercialization. The USDA, FDA, and sometimes the EPA pass judgment on the safety and suitability of such crops. In other countries, similar regulatory approvals must be sought. As a result, the products of agricultural biotechnology that are currently sold for food purposes around the world have been subjected to intensive assessments for their safety for use in foods. The safety assessment of genetically modified foods usually begins with a comparison of the novel food with its conventional or traditional counterpart. With the current generation of genetically modified foods, this comparison has typically indicated that 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 then focuses on the safety of the introduced gene, especially the novel gene product or protein. The current generation of genetically modified foods has been subjected to a thorough safety assessment and is well documented to be safe for its 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. However, no such genetically modified crops have yet been introduced into the marketplace. Furthermore, a regulatory system exists on a worldwide basis to assure that suitable testing will be conducted to ensure the safety of these novel foods for consumers before their introduction into the marketplace.

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