Food Safety Assessment of Genetically Modified Crops

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ABSTRACT

Following the production of the first transgenic plants, health issues concerning the safety of using genetically modified (GM) crops in foods and feeds have been discussed, debated, and evaluated. The main concerns regarding GM foods include toxin or allergen production, changes in nutrient levels, and development of antibiotic resistance. The U.S. Food and Drug Administration established extensive guidelines to address the concerns and ensure the safe commercial introduction of GM crops for use in food or feed. The guidelines were developed through evaluation of scientific data from several disciplines related to genetic engineering and food safety, and consultation with experts from around the world. Foods containing GM crops meeting the FDA guidelines are deemed to be as safe and nutritious as their non-GM counterparts already on the market.

In the 5 yr since the first large-scale commercial harvest, the area planted to transgenic crops in the U.S. has grown to more than 28.4 million ha (more than 71 million acres) in 1999, where approximately 35% of all corn (*Zea mays* L.) and 55% of all soybean [*Glycine max* (L.) Merr.] planted were transgenic (James, 1999). Because soybean and corn are used as ingredients in a wide range of food products, many foods on grocery store shelves currently contain ingredients derived from genetically engineered crops. Barring a consumer backlash, the area planted to transgenic crops will continue to rise, resulting in ever more foods derived from transgenic crops (GM foods) in the marketplace.

Throughout the development and commercialization of transgenic crops, health and food safety concerns have been raised and regulatory procedures established to ensure the safety of GM foods. The main concerns voiced have been questions about the potential of GM crops to:

- Inadvertently introduce toxins
- Introduce allergens
- Change the levels of essential nutrients, and/or
- Compromise antibiotic therapies

Issues of food safety from GM crops came to the fore with a report that a greater number of laboratory rats (*Rattus rattus*) suffered from abnormalities in their small intestines after 10 d of feeding on GM potato diets (*Solanum tuberosum* L.), relative to those feeding on non-GM potato diets (Ewen and Pusztai, 1999). In an extraordinarily unusual step within the scientific field, the same journal that published the paper simultaneously published the rebuttal (Kuiper et al., 1999). The rebuttal made it clear that the potato diet was inappropriate for rats, that similar intestinal abnormalities are well known in rats fed similar diets, and that the sample size (six rats) was too small to draw any conclusions.

The report by Ewen and Pusztai (1999) was seized upon by anti-GM advocates to support their contention that proper regulatory oversight is either lacking or inadequate. This paper was written to address those concerns and to provide information on the assessment criteria and regulatory mechanisms that are in place in the U.S. to ensure the safety of GM foods.

Safety Assurance for Foods Derived from Genetically Modified Crops

Before marketing, GM crops must undergo extensive food, feed, and environmental safety assessments. The Food and Drug Administration (FDA), the U.S. Environmental Protection Agency (USEPA), and the U.S. Department of Agriculture's (USDA) Animal and Plant Health Inspection Service (APHIS) all have jurisdiction for monitoring and regulating the development, testing, and release of GM plants and plant products.

The Federal Food, Drug, and Cosmetic Act grants the FDA authority to regulate commercialization and marketing of most domestic and imported foods in the U.S. market. Under the 1992 Policy interpretation (USFDA, 1992), GM foods and food ingredients must comply with the same standards that apply to other food products. This means that GM foods must be as safe and nutritious as their non-GM counterparts in grocery stores today. The Act places legal duty on developers to ensure that the foods they market to consumers are safe to eat. Transgenic crops containing novel proteins classified as food additives are subjected to additional testing and must receive premarket approval by the FDA before commercialization, while whole foods made from transgenic crops passing GRAS (generally recognized as safe) standards are subject to FDA regulation after commercialization. The FDA has the broad authority to take legal action against developers of any food or food additive that poses a hazard to the public.

The FDA published its 1992 policy to assist developers in addressing food safety and regulatory issues before products reach the market. The policy statement contains a comprehensive "guidance to industry" section that discusses scientific issues for assuring food safety and identifies scientific and regulatory questions that developers must consider to determine if they should consult with the FDA before commercial release of a genetically engineered food crop. Developers are encouraged to consult with the FDA through the Office of Premarket Approval of the Center for Food Safety

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Abbreviations: GM, genetically modified; FDA, Food and Drug Administration; USEPA, U.S. Environmental Protection Agency; USDA, U.S. Department of Agriculture; APHIS, Animal and Plant Health Inspection Service; GRAS, generally recognized as safe; ECSCF, European Commission Scientific Committee for Food.

and Applied Nutrition and the Office of Surveillance and Compliance of the Center for Veterinary Medicine before the commercialization of a new GM variety to ensure that the variety will meet all safety requirements. The FDA requires a full evaluation of a genetically engineered food crop when uncharacterized DNA sequences are used, or when food products may contain significantly altered nutrient levels, different compositions from substances currently found in foods, allergenic proteins, new antibiotic resistance markers, or levels of toxins significantly above those found naturally in edible varieties of the same species.

Inadvertent Synthesis or Increase in Toxins

Many plants produce a number of toxins and antinutritional factors that are thought to provide resistance to natural herbivores (Kessler et al., 1992). In most domesticated crop species, the concentrations of such toxins are so low that they present no health concerns. In other species (e.g., potato), breeders routinely screen new varieties to make sure that toxins are within a safe limit. In some crop species (e.g., cassava, *Manihot esculenta* Crantz), the food product must be cooked or processed to inactivate toxic substances. In GM crop plants, toxins are of concern in any species in which unsafe levels have been found in its lines, varieties or relatives, or if transgenes are derived from such species. In those cases, developers of GM crops must determine that toxins are absent, or present within a safe range, before commercialization of the crop. In addition, a number of groups of proteins present in common foods are toxic or antinutritional. Development of GM crop plants with gene sequences encoding such proteins is not recommended, and if developed, such varieties would be subject to a full food safety review.

Another concern is the potential for *silent* toxin pathways to be reactivated in GM crops due to the genetic engineering process. Plants, like other organisms, have metabolic pathways that no longer function due to mutations that occurred during evolution. Products or intermediates from some of these pathways may be toxins. Questions have been raised as to the potential for genetic engineering to reactivate those pathways. However, a determination was made that the likelihood of such events occurring in food plants with a long history of safe use is remote, and that the potential would likely have been detected during breeding evaluations of the crop and its use for food (reviewed in Kessler et al., 1992). Currently, all transgenic food crops approved for commercialization in the USA (e.g., corn, soybean, potato, etc.) have long histories of breeding and use as foods, and this history can be used to evaluate potential for toxin production. Transgenic crops that have breeding and/or food use histories that indicate a potential for toxin production are subject to a full food safety evaluation prior to commercialization. Similar safety evaluations would be necessary for new crops lacking a long history of breeding and use in food, since the potential for toxin production would be difficult to predict in these crops.

Toxins present in the food supply do not necessarily come from the plant itself, but rather can come from pathogens growing on the plant. A recent report (Munkvold et al., 1999) indicates that corn engineered to resist the European corn borer (*Ostrina nubilalis*) has lower concentrations of the fungal mycotoxin, fumonisin. The corn borer wounds the plant and facilitates infection by Fusarium ear rot (*Fusarium verticilliodes*), hence reductions in insect damage lead to reductions in fungal infection, which ultimately lead to lower levels of fungal toxin accumulation.

Changes in Essential Nutrients

Certain crop plants are major suppliers of essential nutrients to the human diet. For example citrus crops are major sources of vitamin C, carrot (*Daucus carota* L.) is a source of vitamin A, and grain legumes are a source of protein and essential amino acids. Under FDA policy, developers of new food crop varieties, including GM crops, must determine that levels of essential nutrients in the new variety are not significantly different from levels traditionally associated with those crops. If, after reveiw of results from tests, levels of essential nutrients are found to be significantly different in the new crop, the FDA has the authority to disallow commercialization of the crop, or require that foods derived from the crop be labeled to inform the consumer of the altered nutrient content.

Genetically modified crops are being developed with enhanced nutritional qualities, such as increased iron or vitamin A content in edible plant parts (Goto et al., 1999; Ye et al., 2000). In such cases, the transgenic crop could be subject to a full food safety evaluation before approval for commercialization and to labeling requirements following commercialization (USFDA, 1992).

A recent paper by Lappé et al. (1998) reported that GM soybean had lower levels of isoflavones, considered important for human health, than non-GM soybean, which allegedly demonstrates the inadequacy of the current regulatory system. The American Soybean Association promptly reacted by providing documentation that the changes observed by Lappé et al. (1998) were well within the normal range of isoflavone concentrations for soybean (ASA, 1999).

Introduction of Allergens

Food allergies occur in <1 to 2% of the population, and most sufferers have been shown to be allergic to only a few specific proteins in one or two specific foods (reviewed in Hefle et al., 1996; Metcalfe et al., 1996). Eight foods (peanut [*Arachis hypogaea* L.], soybean, tree nuts, milk, eggs, fish, crustaceans, and wheat [*Triticum aestivum* L.]) account for >90% of food allergies worldwide (reviewed in Metcalfe et al., 1996; Taylor and Lehrer, 1996). Food allergies are of major concern, as people with allergies to those foods can exhibit reactions that are sudden, severe, and life-threatening (reviewed in Taylor and Lehrer, 1996).

Guidelines for assessing the allergenic potential of GM crops were established by the FDA following exten-

sive review of research on food allergies and consultation with leading researchers in the areas of food safety, food allergies, immunology, biotechnology, and diagnostics (USFDA, 1994). Under FDA guidelines, developers of crops engineered with transgenes from any of the foods listed above are instructed to demonstrate scientifically that the allergenic substance is not present in the new food.

All known food allergens are proteins, many of which share several features: amino acid sequence similarity to each other, molecular weight between 10 and 70 kDa, glycosylation, acidic isoelectric points, and resistance to heat, acid treatment, proteolysis, and digestion (reviewed in Lehrer et al., 1996; Taylor and Lehrer, 1996). Nevertheless, exceptions exist. Therefore, to ensure that new GM crops do not contain a new allergen, its allergenic potential must be assessed based on:

- The gene source (did it come from a species known to cause food allergy?)
- The crop to be engineered (do foods derived from that crop cause food allergies?)
- The gene and protein sequence (does it share traits with known allergens?)

To aid in evaluation of those criteria, a decision tree strategy was formulated (Metcalfe et al., 1996). If the answer is *yes* to any of the questions posed in the diagram, specific tests and assays are recommended. If allergenic potential is indicated, the FDA will require labeling to inform consumers of the allergenic potential, or to take legal action against commercialization.

To illustrate how the regulatory system works, Pioneer Hi-Bred International scientists introduced a gene from the Brazil nut (*Bertholletia excelsa* Humb. & Bonpl.) into soybean to improve the methionine content of the protein. Because allergic reactions to Brazil nut had been previously documented, skin prick tests, inhibition immunoassays, and immunoblotting were recommended for testing the allergenic potential of the Brazil nut protein engineered into soybean. During the required evaluation process, the protein made by the introduced gene was found to be allergenic (Nordlee et al., 1996), and Pioneer announced it was discontinuing the work with the Brazil nut gene.

Reduced Efficacy of Antibiotics

Genetic engineering of plant cells is an inefficient process, and only a very small percentage of the cells targeted for DNA delivery actually integrate and express the new transgenes. To isolate the transgenic cells from the millions of nontransgenic cells in a tissue, scientists usually link what is known as a selectable marker gene, usually a gene encoding for antibiotic resistance to the transgene(s) of interest. Cells are then grown in a laboratory culture medium containing the antibiotic. Only those cells that have integrated the antibiotic resistance transgene live and grow into plants.

Early in the development of transgenic crops, a concern was raised that the transfer of the antibiotic resistance transgene from plants to pathogens in the environment or in the gut of humans or animals would compromise antibiotic therapy by rendering pathogens immune to the effects of the antibiotic. Accordingly, the 1992 FDA policy statement (USFDA, 1992) and the recently published *Guidance for Industry: Use of Antibiotic Resistance Marker Genes in Transgenic Plants* (USFDA, 1998) specifically discuss the safety evaluation of GM crops and food products containing antibiotic resistance transgenes, by addressing:

- The potential toxicity of the protein encoded by the antibiotic resistance gene
- The potential for the protein to elicit allergenic reactions
- The importance of the antibiotic as a medication
- The frequency of use of the antibiotic
- Whether the antibiotic is orally administered
- The uniqueness of the antibiotic
- The potential for transfer of the antibiotic resistance transgene from plants to microorganisms, and whether such a transfer would enhance the survival of the microorganisms that incorporated the antibiotic resistance gene
- The frequency of antibiotic resistance naturally found in bacterial populations

These guidelines were established following consultation with experts in the fields of microbiology, medicine, bacterial and mycotic diseases, and food safety. Additional confirmation of the guidelines' ability to ensure that GM crops containing antibiotic resistance transgenes are safe was provided by national and international food safety regulatory agencies including the USEPA, the European Commission Scientific Committees for Food and Animal Nutrition, the Nordic Working Group on Food Toxicology and Risk Assessment, and the World Health Organization/Food and Agriculture Organization (ECSCF, 1996; Karenlampki, 1996; USEPA, 1994; WHO, 1993, 1996).

The antibiotic resistance transgene used to develop the GM crop plants currently in the market is the NPTII or APH(3′)II gene, which provides resistance to the antibiotics kanamycin and neomycin by detoxifying them (reviewed in Flavell et al., 1992). A detailed description of the safety assessment of the NPTII gene and its protein product is provided by Calgene (1990), by USFDA (1994) and in the USFDA *Guidance to Industry: Use of Antibiotic Resistance Marker Genes in Transgenic Plants* (1998). In accordance with the criteria listed earlier, the NPTII gene was deemed safe for use (Calgene, 1990; reviewed in Flavell et al., 1992) based on the following:

- No evidence of allergenicity or toxicity could be identified.
- Kanamycin-resistant bacteria are so common in nature that the average human eats 1.2 million bacteria containing the NPTII gene each day, primarily on fresh vegetables.
- Eating food containing NPTII would not compromise the oral use of kanamycin in humans because the protein is rapidly inactivated and degraded in

the gut. Also, NPTII requires ATP to function, and ATP is present in only extremely low concentrations in the digestive system.

- The transfer of the NPTII gene from plants to pathogenic bacteria was highly unlikely to occur, and even if it did, would not increase the amount of kanamycin-resistant bacteria. Each human gut naturally contains 10^{12} kanamycin-resistant bacteria. The worst-case scenario is that eating a tomato (*Lycopersicon esculentum* Mill.) with the NPTII gene would increase the frequency of kanamycin-resistant bacteria in the gut by 0.000001%.

Similar data were presented to demonstrate the safety of the NPTII gene in animal feed and in exposure of soil microorganisms to GM crops in large-scale production scenarios (USFDA, 1998).

Because the DNA used for genetic engineering is produced inside bacteria, a second antibiotic resistance gene, one expressed only in bacteria, is used to permit the preferential growth of bacterial cells containing the engineered DNA. One gene used frequently for the purpose is the *bla* or amp^R gene, which gives resistance to ampicillin. Whenever a gene gun is used to engineer a plant, the *bla* gene can also get incorporated into the plant. Such is the case with Bt maize (*Zea mays* L.) developed by Novartis. However, the ampicillin gene is designed to express only in bacteria, and never in a plant tissue. Because no protein product is produced in plants by the ampicillin gene, its use has been deemed safe (USFDA, 1998).

The conclusion that the NPTII and the ampicillin resistance genes are safe to use in GM crops has also been reached by other scientific panels and regulatory agencies (USEPA, 1994; Karenlampi, 1996; ECSCF, 1996; WHO, 1993). Agreement among independent international organizations regarding the approval and safety of antibiotic resistance markers should reassure consumers of the safety of GM foods containing antibiotic resistance genes.

Nevertheless, the opposite has happened. The presence of the *bla* gene, and fears that it will be transferred from GM crops into pathogens in the environment, was cited as the specific reason why the European Union denied approval of the Novartis Bt maize (Salyers, 1996). Ironically, the ampicillin resistance gene was found and isolated in London, in 1963 (Sutcliffe, 1978), indicating the ampicillin resistance gene is already present in the European environment, and has been since long before GM crops were ever envisioned.

Finally, although evaluation of the safety of antibiotic resistance markers will continue as needed, it may become unnecessary in the future as new, nonresistance based selectable markers are being developed and used in place of antibiotic resistance markers (Ebinuma et al., 1997; Haldrup et al., 1998; Kaeppler et al., 2000).

Summary

Since the creation of the first transgenic plants, health issues concerning the safety of GM foods and feeds have been discussed, debated, and evaluated. Through evaluation of scientific data from several disciplines related to genetic engineering and food safety, and consultation with experts from around the world, the FDA has established guidelines for the safe commercial introduction of food from GM crops. Any such foods that meet FDA guidelines are deemed to be as safe as other foods on the market. Food safety guidelines established using current scientific knowledge are not static, and will continue to be refined as new knowledge is gained in areas related to food safety. For those interested in learning more about safety assessment of GM foods, the following Web sites are suggested as excellent starting points for obtaining further information and references:

http://www.aphis.usda.gov/biotechnology	USDA Biotechnology page
http://www.betterfoods.org/	Better Foods Organization
http://www.ificinfo.health.org	International Food Information Council
http://vm.cfsan.fda.gov/~lrd/biotechm.html	FDA/CFSAN Biotechnology page
http://vm.cfsan.fda.gov/~lrd/fr92529b.html	1992 FDA Policy Statement

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Genetically Modified Crops and the Environment

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ABSTRACT

Genetic modification (GM) of crops provides new crop management options (production traits) and crops with industrial, pharmaceutical, and neutraceutical applications are likely to follow. The environmental benefits and risks of growing GM crops have drawn considerable, often polarized debate. This review seeks a balanced appraisal of environmental issues, and looks at principles associated with several GM production traits. Environmental assessment needs to consider the nature of the introduced trait, in the context of the biology of the plant and the environment it will be grown in (e.g., prospects of gene flow into other species). Interactions with the target ecosystem, including the possibility of cummulative impacts from organisms already released into the ecosystem (e.g., prospects for gene pyramiding) need to also be included in assessments. Current agricultural management practices and ecosystems have their own impacts on the environment, and it is against this background that the benefits and risks of releasing GM organisms should be judged. Before release, data collection on impacts of GMOs is temporally and spatially constrained, so caution must be exercised in decision making. Potential impacts also need to be monitored after release and the post-release monitoring framework needs scope to identify unforeseen impacts. The environmental sustainability of using GMOs will depend largely on wise management practices and monitoring must provide appropriate data to support continuing adaptation of management and regulation of GMOs.

THE use of molecular biological techniques to manipulate DNA and thus alter the make-up of organisms has provided alternative strategies for on-farm management of weeds, pests, and disease (James, 1998). There is also the opportunity to grow crops with industrial, neutraceutical (functional foods), and pharmaceutical (medical compounds) applications. Environmental benefits and risks of GM plants have been debated at length (Brill, 1985; Colwell et al., 1985; Boulter, 1993; Harlander, 1990; Hileman, 1995; Lewis and Palevitz, 1999; Miflin, 1999; Nottingham, 1998; Porter, 1999; Raybould et al., 1999). Nevertheless, adoption of GM crops by the farming community has been rapid. More than 24 million ha of genetically modified (GM) crops were grown in the 1998 season (James, 1998) and about 40 million ha in the 1999 season (ERS, 1999). Continuing environmental concerns challenge further implementation of the technology in a debate that has become polarized and politicized (Glickman, 1999; Serageldin, 1999). It is argued that GM crops place the environment and human health at risk (Greenpeace, 1999; Natural Law Party, 1999) and that life sciences companies responsible for their introduction threaten global food security (Rural Advancement Foundation Int., 1999). The effects of GM corn pollen on larval development of the monarch butterfly (Losey et al., 1999) and the premature release of feeding trial data during GM product development have generated negative public opinion often associated with press coverage of technological controversy (Gaskell et al., 1999; Royal Society, 1999). Suspicion of the intentions of corporate players (Vidal, 1999) has been exacerbated by the provision of inaccurate information to regulators (Coghlan, 1999; Woolf, 1999) and proposals to control seed viability (Oliver et al., 1998). It is in this atmosphere of confrontation and distrust that the impact of GM plants on the environment is discussed. While there is a need to scientifically asses the environmental benefits resulting from the in-

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Abbreviations: GM, genetic modification or genetically modified.