

Starlink Corn: A Risk Analysis

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Modern biotechnology has dramatically increased our ability to alter the agronomic traits of plants. Among the novel traits that biotechnology has made available, an important group includes *Bacillus thuringiensis* (*Bt*)-derived insect resistance. This technology has been applied to potatoes, cotton, and corn. Benefits of *Bt* crops, and biotechnology generally, can be realized only if risks are assessed and managed properly. The case of Starlink corn, a plant modified with a gene that encodes the *Bt* protein Cry9c, was a severe test of U.S. regulatory agencies. The U.S. Environmental Protection Agency had restricted its use to animal feed due to concern about the potential for allergenicity. However, Starlink corn was later found throughout the human food supply, resulting in food recalls by the Food and Drug Administration and significant disruption of the food supply. Here we examine the regulatory history of Starlink, the assessment framework employed by the U.S. government, assumptions and information gaps, and the key elements of government efforts to manage the product. We explore the impacts on regulations, science, and society and conclude that only significant advances in our understanding of food allergies and improvements in monitoring and enforcement will avoid similar events in the future. Specifically, we need to develop a stronger fundamental basis for predicting allergic sensitization and reactions if novel proteins are to be introduced in this fashion. Mechanisms are needed to assure that worker and community aeroallergen risks are considered. Requirements are needed for the development of valid assays so that enforcement and post market surveillance activities can be conducted. **Key words:** allergens, biotechnology, corn, food hypersensitivity, pesticides, risk assessment. *Environ Health Perspect* 110:5–13 (2002). [Online 10 December 2001] <http://ehpnet1.niehs.nih.gov/docs/2002/110p5-13bucchini/abstract.html>

Selective breeding of plants has been one of the most significant achievements of human civilization; it has resulted in major agronomic improvements and in an ability to adequately provide food to large human populations. Conventional plant breeding involves genetic manipulation via crosses of sexually compatible plants and selection for the offspring that have desirable characteristics (such as fruit quantity and quality, pest resistance, and agricultural requirements). More recently, conventional plant breeding has also involved use of technologies such as irradiation that randomly induce mutations that can allow plant breeders to select for new, desirable traits. Biotechnology-based breeding involves direct transfer of specific genetic information in its pure DNA form; unlike radiation, it is a selective process, in that the desired trait and the genetic code that expresses the protein responsible for the trait have been identified and characterized in the parent organism. The process of gene transfer often involves modification of the transferred DNA for better functioning in the new host. Because only a well-characterized segment of DNA is transferred, biotechnology-based breeding is considered to be more precise than conventional breeding, which involves many uncharacterized linked genes. Nonetheless, uncertainties still surround genomic alterations due to the insertion site of the DNA (which is random) and metabolic changes due to the new proteins expressed (which are difficult to predict).

A report of an international academy of sciences panel on biotechnology in agriculture recently concluded that

foods can be produced through the use of GM [genetic modification] technology that are more nutritious, stable in storage, and in principle health promoting—bringing benefits to consumers in both industrialized and developing nations (*1*; p.1).

At the same time, they also said,

public health regulatory systems need to be put in place in every country to identify and monitor any potential adverse human health effects of transgenic plants, as for any other new [plant] variety (*1*; p. 2).

The bacterium *Bacillus thuringiensis* (*Bt*) has been known for decades to elaborate toxins that have insecticidal properties. Preparations of whole *Bt* have been used by farmers as an insecticidal spray for control of lepidopteran and coleopteran insects for at least 30 years (*2*). Most of the *Bt* toxins belong to the Cry (crystal) protein family. Cry toxins are elaborated in crops that contain *Bt* genes; to date such crops include corn, cotton, and potato. One of such proteins is Cry9c; it has been incorporated into a corn variety that has the trade name Starlink (Aventis CropScience, Research Triangle Park, NC). Starlink and other transformed, or transgenic, crops have thus acquired insecticidal properties. They have been termed plant-pesticides, genetically modified pest-protected plants, and most recently, plant-incorporated

protectants (PIP). To date, the economically most important PIP crops are *Bt*-modified plants, or *Bt* crops.

The U.S. Environmental Protection Agency (U.S. EPA) 1998 registration for Starlink was restricted to use as animal feed and excluded consumption as food. In September 2000, the media reported that Starlink somehow had found its way into the food supply (*3*); in a few days, a major food company initiated voluntary recalls (*4*). In early October, the U.S. Food and Drug Administration (FDA) confirmed that Starlink corn was intermingled with corn in the food chain, and by the beginning of November the FDA reported recalls of several products (*5*), with others soon to follow.

In the United States, risks of genetically modified foods are assessed and managed under various statutes that have the intent of assuring that new technologies meet safety standards, which vary according to statute. These legal authorities grew up during different eras and under various congressional committees and agencies, so that they constitute a patchwork that has been held together via a White House “Coordinated Framework on Biotechnology” that was published in 1986 (*6*).

Risks related to PIPs are in the jurisdiction of three agencies, the U.S. EPA, the U.S. Department of Agriculture (USDA), and the FDA. Table 1 illustrates the legal authorities of these federal agencies and the roles of key international authorities. Of note is that the U.S. regulatory process is in flux (as assessed by the number of recent modifications to policies and rules). The three agencies operate under separate statutes and under the oversight of different committees of Congress. They coordinate their efforts under the 1986 White House Coordinated Biotechnology Framework (*6*). The U.S. EPA is the federal agency that evaluates and licenses pesticides under FIFRA [Federal Insecticide, Fungicide, Rodenticide Act (*7*)] and FFDCA [Federal Food, Drugs, and Cosmetics Act (*8*)]. For

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PIPs, the scrutiny of the U.S. EPA encompasses environmental risks and human health concerns, though it is limited to the pesticidal substances (the inserted DNA and the proteins it produces). The USDA, under the Plant Protection Act [PPA (9)], regulates all genetically modified plants that have pest potential. The FDA, given its authority over food safety, oversees all genetically modified plants under the FFDCa that are grown for food or animal feed (8). The FDA also monitors the residues of pesticides in food, thus ensuring their compliance with levels that the U.S. EPA has deemed safe, which are called “tolerances.”

As shown in Table 1, the current legal framework consists of various rules and policies concerning PIP that have been published by the U.S. EPA, the USDA, and the FDA. The USDA issued the first rule, in 1987 (modified in 1997) (10), which requires a review of new plants that the USDA will no longer regulate if the plants are judged to be safe for the environment. Also in 1987, the National Academy of Sciences (11) published a white paper that advised the U.S. government to regulate biotechnology based on risk and not based on the application of a new technology per se. The U.S. EPA proposed a rule in 1994 (12–14), modified in 1997, which was finalized in 2001. It was withdrawn from publication in the *Federal Register* on 20 January 2001 (15). The final regulation was published in July 2001, along with a call for comments pertaining to proposed exemptions that were dropped from the final rule (16). Despite this prolonged rule-making effort, the U.S. EPA gave the first full approval for commercialization of a pest-protected plant in 1994. In 1992 the FDA published a policy statement in which it established a process for voluntary consultation with industry over these products (17); in 2000, it proposed a rule that would require mandatory review of new genetically modified plants (18). In 1999, Congress asked the National Research Council (NRC) to review this biotechnology assessment framework, particularly with respect to PIPs; the NRC published its report in early 2000 (19).

The Starlink episode can be viewed as a severe test of the framework for risk assessment and management of plant-incorporated protectants, both for the U.S. EPA and for the Coordinated Framework. In this paper we analyze these processes in terms of science and regulatory policy to contribute to the dialogue about regulation of genetically modified crop plants. Such an analysis may be useful for suggesting areas where further reform of biotechnology regulation might be considered. This is particularly timely in that the three agencies, the U.S. EPA, the FDA, and the USDA, are currently in a process of

reexamining their policies on biotechnology plants. Because the major controversies in the case of Starlink were over adequacy of protection of human health, this paper concerns only the human safety aspect of assessing and regulating biotechnology.

Chronology

The saga of Starlink corn occurred over a 5-year period between 1997 and 2001 (Table 2). However, it should be noted that the first strain of *Bt* corn to be marketed in the United States was based on Cry1Ab and was approved for human consumption in 1995 (20). Until spring 2001 only, another Cry toxin (Cry1Ac) was approved thereafter as an active ingredient of transgenic corn intended for human consumption (21). Although the technology to induce endogenous production of *Bt* in plants was then novel, the U.S. EPA was familiar with *Bt* toxins because it had issued several registrations for *Bt* sprays and extracts. From the standpoint of human health, the U.S. EPA has considered *Bt* sprays to be safe—so safe that it has exempted them from the requirement of a tolerance (a standard for a maximum allowable residue limit on food) and thus exempted them from FDA enforcement requirements. This means that foods containing residues of *Bt* would not be considered by the FDA to be adulterated, even though no tolerance was established. All of the *Bt* plant registrations issued by the U.S. EPA have followed suit; that is, in no case has a tolerance been required (21).

In 1997, Plant Genetic Systems, a company that is now part of Aventis CropScience, applied to the U.S. EPA for approval of a registration under FIFRA for Starlink corn for

food use. At the same time, it petitioned the U.S. EPA for an exemption under the FFDCa from the requirement of a tolerance for residues of Cry9c in food and feed (22). The U.S. EPA published the petition for tolerance exemption for public comment and received no comments. Later that year, Aventis modified the application for registration to request approval of Starlink for animal feed but not for human consumption; this application was granted in 1998, and the tolerance exemption was published as a final rule (Table 2) (23). In 1999, the U.S. EPA denied an application to expand the registration to include food uses (24). At that time, the U.S. EPA asked its Scientific Advisory Panel (SAP) for advice on the review of Starlink. Meanwhile, the USDA approved (“unregulated”) Starlink in 1998, after receiving 2,271 letters from farmers in support of Starlink (25,26). In the same period, the FDA reviewed the other food safety aspects and considered its consultation with the company completed (18).

The regulatory history of Starlink before its occurrence in the food supply suggests that the U.S. EPA had decided to proceed with caution. Such caution is reflected in Starlink being the first *Bt* pest-protected plant to receive an animal-feed-only registration. Once Starlink residues were detected in the food supply, in the fall of 2000, the company withdrew the registration. At the same time, it petitioned the U.S. EPA for a time-limited approval for food uses to make legal the Starlink corn that was inadvertently present in the food supply. These efforts continued into 2001 with additional data submittals by Aventis. In July 2001, the U.S. EPA FIFRA SAP once again was asked for

Table 1. U.S. federal agencies and selected international entities with legal authority in regulating genetically modified plants.

Agency	Authority	Specific regulations	Responsibility
White House	Executive	Coordinated framework	Coordination of federal activities
USDA	PPA	7 CFR 340	New pests; environmental impact
U.S. EPA	FFDCa, FIFRA	FFDCa rules FIFRA rules	Plant incorporated protectants (health and environmental risk)
FDA	FFDCa	1992 Statement of policy 2000 Proposed policies ^a	Whole foods Risk to human health
EU ^b	EC Treaty (art. 100a)	Directive 90/220/EC ^a	Environmental risks
EU ^b	EC Treaty (art. 100a)	EC 258/97 EC 1139/98 Reg. 49/2000 ^a	Food risks and labeling
UN	Biodiversity Convention	Biosafety Protocol	Interboundary movement of LMOs
Codex	FAO	In process	Labeling; human health

Abbreviations: EC, European Community; EU, European Union; FAO, U.N. Food and Agriculture Organization; FFDCa, Federal Food, Drugs and Cosmetics Act; FIFRA, Federal Insecticide, Fungicide and Rodenticide Act; LMO, living modified organism; PPA, Plant Protection Act.

^aNew rules proposed. ^bThe decisions are taken by an individual member state regulatory agency and valid throughout the union if no member state objects; in the case of objections, they are taken at the European level and involve the European Commission.

advice. At this stage, the SAP concluded that it could not identify a safe level for Cry9c in the food supply. It recommended that the U.S. EPA continue efforts to remove Starlink corn from the food supply (27). At this stage, it appears highly unlikely that there will be an approval for Cry9c in food.

Risk Assessment

In the case of PIPs, as for other pesticides covered under the FFDCa, the legal standard for safety is “a reasonable certainty of no harm” under the Food Quality Protection Act (28). In other words, the U.S. EPA must be able to make a judgment that no one will be harmed by the occurrence of the pesticide residue on food. Product-specific data are developed by companies (registrants), generally under protocols designed by the U.S. EPA in consultation with the scientific community. These protocols generally rely on both *in vivo* and *in vitro* toxicology testing. Risk assessments are conducted by the U.S. EPA. For a chemical pesticide in food, the U.S. EPA generally assesses acute toxicity, mutagenicity, reproductive and developmental toxicity, subchronic toxicity, chronic toxicity, neurotoxicity, and oncogenicity. In the case of PIPs, whose pesticidal properties are conferred by DNA and proteins, the U.S. EPA has tailored the hazard identification process to the characteristics of these compounds, thus reducing the testing to the safety concerns that the U.S. EPA has concluded are warranted.

Both the U.S. EPA and the FDA have concluded that DNA, as a food component, is inherently safe, and they therefore have not subjected DNA to testing. Although proteins are a normal component of food, there are many proteins that are toxic. These include bacterial toxins (e.g., botulinum

toxin), components of snake poisons, and plant toxins. The U.S. EPA and the FDA consider that known toxic proteins in food act via acute mechanisms at very low dose levels; chronic health impacts, or other adverse effects, are not considered likely. Toxic proteins are regulated with a zero risk approach by the FDA, meaning that the policy is that no tolerance may be established (29) because of their great potency. Thus, in assessment of PIPs, the U.S. EPA has required companies to conduct high-dose acute toxicity assays. This maximum hazard dose approach has been endorsed by the U.S. EPA SAP (30).

Beyond acute toxicity, the U.S. EPA is also concerned about the potential for adverse immune effects of proteins, especially food allergy. Unfortunately, proteins that are allergens do not have properties that completely differentiate them from other proteins. Allergens interact with the immune system in a two-step process: sensitization and allergic reactions. Allergic reactions are associated with a spectrum of adverse effects. In young children, food allergy can cause chronic diarrhea and adversely impact nutritional status and growth. Food allergy can also cause acute allergic reactions, called anaphylaxis. Severe anaphylaxis can cause shock (anaphylactic shock)—severe respiratory and cardiovascular symptoms that can result in death. One study estimated that as many as 8,300 cases of anaphylactic shock from all causes occur in the United States each year, and that about 415 result in death (31). Bock et al. (32) estimated, based on a study by Yocum et al. (33), that each year 29,000 food allergy-related anaphylactic episodes (including anaphylactic shock) occur in the United States, with 150 associated deaths. The prevalence of food allergy in the general population is not known precisely; it is estimated

at 1–2% in the general population and up to 5% in the pediatric population (34).

PIPs are of concern from the standpoint of allergenicity for two reasons. First, genetic modification could theoretically create unintended changes in the plant, which could result in expression of new allergens or in increased expression of endogenous allergens. This potential hazard has not been a concern for Starlink. Second, the protein, or proteins, purposely expressed as a consequence of genetic modification could be allergenic. In the first scenario, the protein could be a known allergen derived from another food. The development of a more nutritious, methionine-rich soy variety was halted when immunologic assays revealed that human sera from Brazil nut-allergic patients were reacting to the new Brazil nut protein that was introduced into the soy (35). Under the second scenario, the protein is novel to the food supply. In that case, no testing material would be available from human populations to identify the allergen before it is introduced into the food supply.

Consequently the theoretical potential risk of allergenicity of biotech crops has been under consideration since 1992 (17). In 1994, the FDA, the U.S. EPA, and the USDA sponsored a meeting that developed tentative guidelines for allergenicity assessment. In 1996, a science consensus paper (36) defined an allergenicity assessment decision tree that has formed the core of the approach used by the FDA and the U.S. EPA. Figure 1 illustrates the part of the decision tree that is relevant to the identification of a new allergen in the food supply. Using this framework, assessment of allergenicity is addressed with stability studies and amino acid sequence analysis. It is generally believed that proteins easily degraded by gastric juices or by food processing are less likely to cause sensitization and allergic reactions, although exceptions exist (37). Based on this rationale, studies of simulated gastric fluids and heat stability are used to determine whether the protein would be available to interact with the immune system (37). Amino acid sequence analysis is used to search protein databases for sequences that are similar to known allergens, or that share epitopes with known allergens. Proteins that fail to produce these matches are considered less likely to be food allergens. However, the U.S. EPA's FIFRA SAP has identified some uncertainties in such analyses (30).

One issue that the U.S. EPA does not address is the potential for allergy via the inhalation route of exposure. Allergens in grains and other foods are known to cause occupational asthma or rhinitis (38), particularly in workers in silos and bakeries. Cases of occupational allergies to *Bt* products have

Table 2. Key events in the regulation of Starlink corn by EPA and FDA.

Date	Event	Authority
August 1997	Aventis ^a petition to register Starlink	FIFRA
September 1997	Aventis ^a petition for all uses	FFDCA
November 1997	Aventis ^a petition for feed use only	FFDCA
April 1998	EPA: Feed use only granted (temporary)	FIFRA
May 1998	EPA: Feed use only (permanent)	FIFRA
August 1998	EPA: Starlink registered ^b	FIFRA
April 1999	Aventis ^a new petition for all uses	FFDCA
February 2000	First Starlink SAP	FIFRA, FFDCA
April 2000	EPA: Registration updated and extended	FIFRA
September 2000	Friends of the Earth finds Starlink in tacos; voluntary recalls	
October–November 2000	FDA recalls various foods	FFDCA
October 2000	Aventis ^a suspends registration	FIFRA
October 2000	Aventis ^a petition on time-limited use in foods	FFDCA
November 2000	Second Starlink SAP	FIFRA, FFDCA
April 2001	Aventis ^a petitions for tolerance for Cry9c	FFDCA
June 2001	CDC report to FDA on adverse event reports	
July 2001	Third Starlink SAP	FIFRA, FFDCA

SAP, FIFRA Scientific Advisory Panel.

^aFor simplicity, the petitioner is always referred as Aventis, although the first petitions were submitted by Plant Genetic Systems. ^bUnder the conditions that Aventis would follow USDA NC-205 refuge guidelines for all Cry9c, that the field corn be used for animal feed or industrial use only, total acreage would not be more than 120,000, and the registration would expire on 30 May 1999 (47).

been reported and confirmed in a recent study of farm workers (39), but only a small fraction might be attributable to Cry proteins. Also, bacterial enzymes used in detergents reportedly caused adverse reactions in occupational settings (40,41) and among consumers (42) before preventive measures were introduced. Soy proteins released to the air at grain-loading docks caused community outbreaks of asthma in Spain in 1985–1986 (43) and in New Orleans (44). Stability with processing and digestion would not be relevant to assessment of the potential for inhalation allergenicity.

Proteins may also have antinutritional properties; they may decrease absorption of nutrients. An example of this is lectins that are present in many plants and are harmful unless cooked. The U.S. EPA and the FDA are aware of the existence of such proteins. The U.S. EPA assumes that new proteins with antinutritional activity will have sequences that are similar to known antinutritional proteins.

Recently, the NRC reviewed the regulatory framework for pest-protected plants by the U.S. EPA, the FDA, and the USDA (19). The report's conclusion was that, while there is no evidence that genetically modified foods are unsafe, there are three theoretical human health concerns that need to be addressed: allergenicity; toxic compounds in plants; and risk from chronic exposure. The NRC called for long-term feeding studies of animals to assure that chronic toxicity and nutritional changes are not occurring.

The U.S. EPA's health evaluation of Starlink was exceptionally lengthy as compared to other PIPs because it spanned 1997–2001, with four rounds of data submissions from Aventis (1997–1998, 1999, 2000, and 2001) (45). The analysis of the U.S. EPA's risk assessment will follow the conventional risk assessment model (hazard identification, exposure assessment, dose–response characterization, and risk characterization). Through the examination of the application of this framework, the most relevant underlying assumptions and information gaps will be discussed.

Hazard Identification

Identity of the agent. Cry9c, the *Bt* toxin present in Starlink, protects corn from lepidopteran species and particularly from the European corn borer, *Ostrinia nubilalis* (Huber), a major pest of corn and other agricultural crops. The commercial product Starlink contains in its genome a modified sequence of the *cry9Ca1* bacterial gene, originally derived from strain BTS02618A of *Bacillus thuringiensis* serovar tolworthi, which was isolated from grain dust in the Philippines (46). The bacterially encoded

protoxin is a 129.8-kDa protein; specific modifications of the *cry9Ca1* gene inserted in Starlink result in a corn-expressed Cry9c protein that is C-terminal truncated (elimination of all the amino acids following position 666), that is N-terminally truncated (all amino acids before position 43 substituted with two amino acids, alanine and methionine), and that has a replacement of arginine with lysine at amino acid position 123 (47). The bacterial protoxin is normally cleaved to a 68.7-kDa active toxin whose toxicity is normally reduced by further trypsin digestion to an inactive 55-kDa fragment. The substitution [arginine to lysine (46)] reduces the susceptibility of the 68.7-kDa toxin to trypsin cleavage. Therefore, the Cry9c protein expressed in corn is the active 68.7-kDa toxin with enhanced stability to trypsin digestion. This protein and its coding DNA are what the U.S. EPA considers the active ingredients of Starlink and therefore are covered as a PIP. The modification process is summarized in Figure 2.

The concentration of the Cry9c protein in the Starlink corn plant is lower than in bacteria and extraction is more difficult, making it difficult to produce protein for toxicity testing. For this reason, the U.S. EPA accepted microbially produced trypsinized Cry9c as a test substance. Trypsinization is essential as it yields a fragment of 68.7 kDa similar to that expressed in plants. Different post-translational modifications in the two systems (bacteria and plant) could lead to different characteristics. The substitution of the microbially produced Cry9c was validated by comparative analyses, including SDS-PAGE gels, Western blots, N-terminal amino acid sequencing, glycosylation tests, and insect bioassays. Based on these assays, the U.S. EPA concluded that there were no biologically relevant differences between the plant Cry9c and the microbial Cry9c produced for the assay and that the latter could be used for further testing. However, later data produced by Aventis, as discussed below, raise doubts about whether such equivalence should be accepted for allergenicity assessment, because Cry9c may be glycosylated in plants but not in bacteria.

Acute toxicity. There is a long history of safe use of *Bt* sprays and other agricultural preparations (2). Apparently, no confirmed reports of adverse effects due to Cry proteins have emerged (48) and recent data support this notion (49). *Bt* also has a relatively benign mode of action, in terms of risks to humans. Cry proteins, once ingested by insects, bind to brush border membrane receptors and insert into the membrane-forming pores, eventually disrupting the cells and causing massive leakage (2). These receptors do not seem to exist in mammals (48).

In compliance with U.S. EPA requirements, Aventis supplied an acute, single-dose toxicity study that found an LD₅₀ (median lethal dose) > 3,760 mg/kg in mice. In the study, transient weight losses were seen in three females and in no males. This adverse effect had been seen in other Cry protein studies and was considered insignificant. Moreover, Aventis showed that the protein did not display significant homologies to known human toxins in three different protein databases. These searches found homologies only to other *Bt* proteins.

Allergenicity. The biochemical characteristics of Cry9c created challenges for the U.S. EPA in applying allergenicity assessment principles that had been used to support the safety of other pesticidal proteins. Consequently, there was a rapid evolution in the nature of the experimental evidence that the U.S. EPA and Aventis employed in the effort to evaluate the potential for allergenicity. The original submission (22) relied on stability studies and sequence analysis. These data did not rule out the possibility of allergenicity of Cry9c. The *in vitro* digestibility study showed that Cry9c is stable for 4 hr when exposed to pepsin digestion at pH 2.0. Cry9c is also stable to trypsin because, as noted above, it has been modified for increased proteolytic resistance. The protein was also found heat stable for 10 min at 90°C. Therefore, it was concluded that Cry9c was likely to survive processing and digestion and, as a consequence, to have the potential to interact with the immune system. The sequence analysis did not show any homology with known allergens. However, on the basis of its relative stability, the U.S. EPA restricted Cry9c to animal-feed use (23). Later, it was recognized that Cry9c may be glycosylated in plants; many believe that glycosylation is a feature of many food allergens (50) and that glycosyl groups contribute substantially to allergen binding (51,52). Retrospectively, the equivalence of

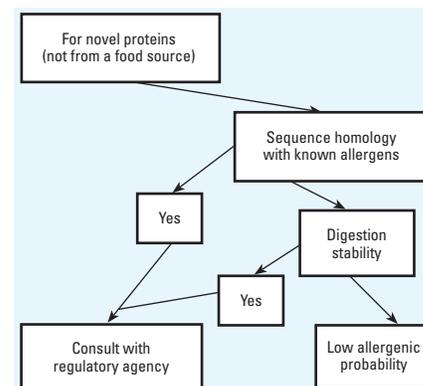


Figure 1. 1994 FDA/U.S. EPA decision tree for allergenicity assessment of novel proteins that do not come from a food source (36).

trypsinized microbially produced Cry9c to the plant product seems to have been questionable; in fact, bacterially produced recombinant allergens sometimes cannot be validated, and eukaryotic systems may have to be used for their production instead, at least for aeroallergens (53).

In 1999, Aventis submitted several new studies. One of them provided evidence, that, when assayed against the sera of corn-sensitive patients, Starlink was no more allergenic than conventional corn (45). The U.S. EPA concluded that people with no prior exposure to Cry9c would not be sensitized to Cry9c, nor was there any reason to expect that those persons with allergies to native corn proteins would become sensitized to the bacterial protein Cry9c. Thus, this study was not considered relevant to the assessment of risk. At the same time, the company provided an animal study that showed that Cry9c can produce allergic reactions in the Brown Norway rat (45). However, the specificity of this model for allergenicity prediction has not been determined and, therefore, the U.S. EPA did not consider the data for its assessment. A third study indicated that Cry9c could be retrieved from the blood of animals fed the pure protein (45), validating the earlier conclusion that the protein could survive processing and digestion. A fourth human study consisted of a series of letters from workers exposed by inhalation and possibly by dermal contact to

Starlink corn (45). This study was not considered relevant by the U.S. EPA and the U.S. EPA's SAP, particularly because workers reported no allergic reactions, whereas it is expected that 30% of a random sample of the population would report some allergic reactions (54). In summary, this new set of data did not cause the U.S. EPA to change its assessment of hazard.

In November 2000, the U.S. EPA convened a panel of the SAP to review its assessment of the potential for allergenicity of Starlink corn. The U.S. EPA had refined its assessment to address separately the *a priori* probability of a protein to be an allergen and, separately, the probability of sensitization and allergic reactions in the population due to exposure to that protein. In terms of *a priori* allergenic potential, the SAP provided a new rationale for assessing the potential allergenicity of proteins, though it was cautious about the validity of these criteria. The criteria are resistance to acid treatment and to protease digestion, molecular weight range, glycoprotein, immunologic responses in the Brown Norway rat (an element considered by the SAP, and not by the U.S. EPA), and presence of the protein in the bloodstream of the rat (54). (Table 3 is a complete list.) Based on these criteria, the SAP concluded that Cry9c had a medium likelihood of being an allergen. Although difficult to quantify, the potential for allergenicity of Cry9c seems to exceed that of other Cry proteins.

Exposure Assessment

In October 2000 Aventis presented an exposure assessment for Cry9c (45). In response to it, the U.S. EPA produced its own revised assessment. Exposure was estimated according to standard methods based on the USDA national food consumption surveys and Monte Carlo modeling; these methods allow an estimation of distributions of exposures to corn in various products in the diet across the population and for various age groups. The estimates of exposure to Cry9c protein in the food supply also were driven by assumptions related to patterns of use (animal feed vs. human food), percentage of crop planted with Starlink corn, and degree of blending

Table 3. Criteria adopted by the U.S. EPA SAP for assessment of allergenic potential of proteins (November 2000).

Criterion	Cry9c
Protease digestion stability	Yes
Acid treatment stability ^a	Yes
Immunologic response in BNR ^a	Yes
Bioavailability in bloodstream of BNR ^a	Yes
Probable glycosylation ^a	Yes
Molecular weight of allergens (10–70 kDa)	Yes

BNR, Brown Norway rat.

^aThe method to perform this assay has not been standardized.

with other corn. Under the assumptions of limited compliance and partial mixing, the U.S. EPA concluded that the exposure to the most highly exposed population (Hispanic children, 7–12 years of age) at the 95th percentile would have been 1.6 µg/day in 2000 and 2.6 µg/day in 1999 (45).

Assessment of exposure to food allergens is particularly challenging, in that adverse events are the result of a biphasic process. In the first phase, the immune system encounters the allergen and becomes capable of responding to it (sensitization). Later, the sensitized immune system encounters the allergen and allergic reaction is triggered. The doses and the frequency of exposure required for each process are not easily determined (54), although it seems that sensitization may require higher doses than the low doses that can cause allergic reactions (45). In practice, it is easier to identify doses that trigger allergic reactions than allergic sensitization.

Nonfood exposures were not included in the risk assessment because Aventis and U.S. EPA (55) held the view that, because Cry9c is expressed in plant cells, exposure through inhalation and dermal contact would be negligible. As was mentioned earlier, this is a questionable assumption. In addition, the assessments did not address the potential for cumulative exposure to other *Bt* proteins along with Cry9c. It is known that many allergens show cross-reactivity with similar proteins, even when exposure happens through different routes (56). If Cry9c were an allergen, would it cause sensitization by cross-reactivity to other *Bt* proteins as well, thus making other safe products unsafe?

In conclusion, the available data would indicate that the exposure to Cry9c in food, under what the U.S. EPA considers reasonable assumptions related to mixing with other grain and food use patterns, would be 2.7 µg/day for Hispanic children 7–12 years of age (1999; 1.6 µg/day in 2000), the group with the highest expected exposure. According to the U.S. EPA, under a “high end” scenario, this would be 17 µg/day for the 95th percentile of 7- to 12-year-old Hispanic children (in 1999; 14 µg/day in 2000) (45). This exposure might even be lower if processing of corn reduced Cry9c content (which is likely). This level of exposure was characterized by the November 2000 SAP as low.

Dose–Response Assessment

There are no standard methods for doing predictive modeling of dose response for potential food allergens. There might be thresholds for sensitization to an allergen and for triggering allergic reactions in sensitized individuals. Sensitization is difficult to observe in human populations; therefore, attempts have been made to establish thresholds for allergic

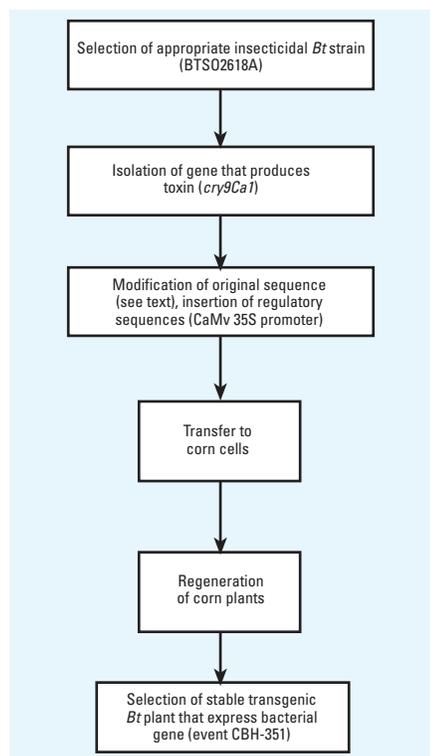


Figure 2. Schematic diagram of the process used to create a transgenic *Bt* plant, with simplified Starlink-specific information in parentheses.

reactions in humans, but with some limitations (57). In the absence of models for Cry9c, it has been suggested that the peanut allergen be used as a basis for exposure comparison for Cry9c: peanut allergy is prevalent in the United States and is known to cause severe reactions (34). However, the U.S. EPA has yet to establish a methodology for dose–response assessment.

Risk Characterization

In November 2000, the FIFRA SAP stated:

The likely levels of the Cry9c protein in the U.S. diet provide sufficient evidence of a low probability of allergenicity in the exposed population (49, p. 24).

It is difficult to establish whether the Cry9c protein is allergenic to humans by both the oral and inhalation routes of exposure. Cry9c has some properties of allergens; specifically, it is probably available for immune system interactions. The levels in foods are probably low, and the efforts of Aventis and other producers to remove the product from the food supply probably made the exposure even lower. However, there is little established science to evaluate the significance of these exposure levels. The inhalation route of exposure has not been evaluated at all, and the U.S. EPA therefore did not characterize risks of workplace exposures. On the other hand, there is no proof that Cry9c is allergenic or that it poses a significant risk at all.

In conclusion, the allergenic risks are highly uncertain. It is clear, however, that Cry9c poses a risk, although this may not be significant. Given that some exposure information was made available, the hazard-only approach applied to other PIPs did not apply here. However, the absence of methods for dose–response modeling made it difficult to make final judgments about the safety of Cry9c. This situation is reflected in the way the U.S. EPA has managed Starlink.

Risk Management

The risk management effort for Starlink involved four major elements: regulatory action, stakeholder involvement, enforcement, and surveillance.

Regulatory Actions

The U.S. EPA carried out regulatory action under both FIFRA (7) and FFDCA (8). As mentioned above, in 1998 the U.S. EPA granted an approval for animal feed use of Cry9c (58). As it often had done for chemical pesticides, the U.S. EPA required market segregation so that the Starlink corn would not inadvertently enter the food supply (23). Other conditions related to the registration were buffer zones, limits on total acreage, and resistance prevention measures. The first

condition, buffer zones, was imposed to ensure segregation. Theoretically, corn grown within 660 ft of Starlink corn could produce the toxin because of cross-pollination. Therefore, a 660-ft buffer zone was required to segregate Starlink corn from other corn varieties. The second provision related to total acreage planted as Starlink; it could not exceed 2.5 million acres during the registration period (years 2000–2001). Other provisions were related to ecologic risks.

Under the FFDCA (8), the U.S. EPA exempted Cry9c from the requirement for a tolerance for residues that might appear in animal feed, as well as in meat, poultry, milk, or eggs resulting from animals being fed with Cry9c corn. These exemptions were supported by the SAP's opinion that amounts of the proteins in milk would be negligible, and probably thus insufficient to cause allergic reactions (54).

Stakeholder Involvement

Broad input by stakeholders is important in ensuring wise decisions and peer review by the broader scientific community. The U.S. EPA sought to involve stakeholders, and especially scientists, in many phases of the decision-making process. Stakeholders included the producer of Starlink (Aventis) and its competitors, farmers, food processors, consumers, public interest and environmental groups, and organic farmers. During the initial review and approval process, the product received relatively little attention from most of these groups. For example, no comments were filed when the U.S. EPA published the petition for a tolerance exemption (23). After the second petition, this changed dramatically, resulting from the appearance of Starlink in the food supply. Other stakeholders include allergy patients, allergists, and other medical experts. With the exception of the physicians who are on the SAP, this community is not yet involved. At the same time that there are concerns, there is also hope by some allergists that biotechnology might be a means to reduce food allergenicity in the future by replacing allergenic proteins in food with benign ones (59).

Because the process of establishing and creating exemptions for tolerances is rule making, formal notice and comment is required. The U.S. EPA went beyond requirements to convene three SAP meetings to evaluate this product and the attendant regulatory decisions. Stakeholders were invited to submit comments for review by the panel as part of its deliberations. In summary, the U.S. EPA process does seem to have been inclusive.

Enforcement

Enforcement of the pesticide law is a complex process involving two federal and various state

regulatory agencies. FIFRA (7) is enforced by both the U.S. EPA and the state lead enforcement agencies (usually the state agriculture and sometimes the state environmental agencies). However, state regulators do not routinely track shipments of corn varieties. The FFDCA (8) is enforced by the FDA via monitoring of the food and feed supply for pesticide residues. Food with residues in excess of tolerance is adulterated and is not allowed to be sold. In the case of Starlink corn, however, there was an exemption from the tolerance for Cry9c, and therefore there was no FDA monitoring for the pesticide. In addition, no validated analytical assay was required (23). The U.S. EPA accepted a plan proposed by Aventis (60) that relied on the company for enforcement and tracking sales and units of Starlink corn. However, there was no clear mechanism for the U.S. EPA to assure that Aventis was doing this; the press reported that there might have been gaps in this effort (61).

In the case of Starlink corn, the requirement to plant the corn only for animal feed was difficult to enforce in the absence of FDA monitoring, which was in turn a consequence of the tolerance exemption for Cry9c. In September 2000, a consumers' group, Friends of the Earth (62), held a press conference to announce its finding of Starlink corn in food from a supermarket. What ensued was a crisis of large proportions for consumer confidence, the food industry, the corn growers, the biotechnology industry, and government regulators. Following this report, the FDA ascertained the presence of Starlink in a variety of food products containing corn, including taco shells (5). Initially, the FDA faced the difficulty of developing and validating an assay to detect the presence of Starlink corn in foods, on an emergency basis. A DNA-based detection method first had to be developed and validated and the basis for recalls was the presence of Starlink-associated DNA (5). Since that time, ELISA-based methods have been developed that detect the Cry9c protein (63).

Were the buffer zones honored, or did they fail to achieve their intended purpose? There are strong indications that Starlink corn pollinated other varieties, based on monitoring of food items that contain not yellow Starlink corn but other varieties of white corn. At this time, it is not clear if this was due to a breakdown in enforcement or a misunderstanding of the ability of corn to pollinate over a long distance.

Surveillance

Surveillance is an important tool, not only to support enforcement but also to inform the risk assessment process. There are several ways that surveillance can be conducted for a product such as Starlink corn, including monitoring

for presence of the protein in the food supply and surveillance for potential human health impacts. Collection of usage data was mandated by the registration (58). The FDA did not monitor the food supply for the Cry9c protein because the U.S. EPA granted a tolerance exemption, as described above.

The FIFRA SAP in February 2000 was asked by the U.S. EPA to address the issue of surveillance for broader health impacts of Cry9c. The SAP recommended animal-, occupational-, and population-based surveillance both to increase the knowledge base for considering approval and to identify adverse impacts (54). However, they did not have specific recommendations for how such surveillance would be conducted, and it is clear that there are clinical and epidemiologic challenges in finding cases of food allergy due to a novel allergen in specific varieties of corn that compose only a fraction of the corn in the food supply.

The FDA has received reports about alleged new cases of allergy to corn, which were prompted by reports in the press. The FDA and the U.S. Centers for Disease Control and Prevention (CDC) investigated these complaints. The FDA developed a new ELISA to detect Cry9c antibodies in serum. The CDC conducted an epidemiologic investigation that included reviewing the adverse event reports; administering questionnaires to all people who experienced adverse health effects and manifested signs and symptoms consistent with allergic reaction; obtaining relevant medical records; and collecting serum samples for temporary banking. The investigation concluded that 28 people had experienced apparent allergic reactions in conjunction with reporting eating corn products that may have contained the Cry9c protein. The FDA assay method found that none of the CDC-submitted samples reacted in a manner consistent with an allergic response to the Cry9c protein, and the CDC concluded that there was no evidence that the reactions people experienced were associated with hypersensitivity to Cry9c protein (64). The U.S. EPA SAP reviewed these data in July 2001 and concluded that

the technical approach for the detection of Cry9c protein and antigen-specific IgEs is limited and cannot resolve the issue of the presence or absence of Cry9c-specific IgE in the serum of individuals reporting adverse reactions after eating corn (27; p. 33).

Societal Impacts

It is too early to estimate the full impact of the Starlink food episode. Certainly, to date, one of the main impacts of the episode has been to raise the level of awareness of the American public about the presence of genetically modified foods in the food supply.

The economic impacts are difficult to estimate. The USDA has confirmed that U.S. exports of corn have suffered. In addition, a recent newspaper article stated that

In all, the [Aventis] official said, more than 28,000 truckloads, 15,000 rail cars and 285 barges of corn tested positive for Starlink (65; p. 417).

Although it is known that recalls can damage companies and their brand names, it is difficult to establish to what extent corn and corn products had losses in the market during this episode. The massive testing effort to exclude Starlink contamination had significant costs, and Aventis estimated its cost to buy back Starlink corn from farmers to exclude further contamination at around \$100 million (66). No doubt, there will be other costs associated with lawsuits, rulings, and settlements (67). It is clear that the entire food industry in the United States will now take a more cautious approach to these products in the future to avoid the market dislocation that occurred in this episode.

Federal regulatory agencies may take a more precautionary approach in the future, in terms of granting approvals and enforcing limits, and certainly, the case for genetically modified food labeling has been advanced. "Split approvals," or feed-use-only approvals for biotechnology products, seem to be another casualty of Starlink; some have argued that segregation has been shown as impossible (65). However, trade needs and voluntary labeling schemes suggest that segregation will remain with us, and that enforcement might become tougher. From a risk perspective, it is apparent that the difficulty in containing contamination makes management difficult and places a higher burden on the risk assessment because our capability to remediate does seem to be low and costly.

Scientific Implications

Numerous questions remain unanswered about Starlink corn. These are due to gaps in our knowledge about areas such as food allergies. Inherent in the risk assessment process is a great deal of uncertainty. However, in the case of Starlink corn, the limitations in our ability to predict and model allergenicity of new proteins pose unusual levels of uncertainty for decision makers. Another issue that Starlink has highlighted is that of the importance of controlling cross-pollination (65). Assessment of this factor is important for exposure estimates (45); it is also important to predict our ability to manage a risk.

A public health and scientific question relates to the analytic method used to assay transgenic crop contamination, and particularly whether DNA or protein should be

assayed. Because DNA seems to be safe and its degradation pattern is probably different from that of proteins, it would seem logical that the protein, the potential allergenic hazard, would be assayed. In fact, the exposure assessments for Starlink concerned Cry9c concentration. However, European regulations also prescribe DNA-based monitoring (68) as a basis for the European biotechnology labeling efforts, which reflect consumer concerns about genetically modified organisms. This has some importance in driving the technology because PIPs could be developed that express little or no protein in the edible parts of the plants, yet would contain the DNA sequences in every cell.

In the case of Starlink, risks to children have been addressed in the exposure assessment, and the exposure of Hispanic children, the most highly exposed group, has been estimated. Children are particularly at risk of food allergy and have extensive exposure to corn (49). Perhaps future assessments should look at other exposures and at the way children respond to allergens.

It is clear that there is no adequate occupational or general population-level surveillance for potentially allergenic novel crops. For human health surveillance, most allergists are not aware of the allergenic risk of biotechnology crops, clinical tests are not readily available, and there is no specific program for monitoring either the general population or occupational groups. Although the FIFRA SAP recommended that the U.S. EPA consider establishing surveillance of animals, it is not clear how the U.S. EPA would do this. In short, the question of surveillance has raised a number of interesting questions and has provided no easy answers.

Risk Communication

Risk communication has been difficult because the risks are so uncertain. Of the many observers, few have been on the fence about the risks. On the one hand, Thomas Hoban wrote in the *Washington Post*:

But what's the startling discovery the alarm-raisers have made? Hold onto your seats, folks: Our corn, it seems, has been contaminated by—corn! For all its ominous overtones, the StarLink incident has very little to do with science and safety (69; p. B2).

In contrast, an editorial published in *USA Today* stated:

Two scientific reviews have failed to produce conclusive evidence that eating the corn, called StarLink, won't produce allergic reactions in infants and young children (70; p. 18A).

In reality, the health impact is not clear. Given the scientific uncertainties, it is probable that better communication can be achieved only as the information gap narrows.

Where communication should be important is in involving the public and stakeholders in defining goals for risk assessors and managers. In fact, it is not clear how society values outcomes such as sensitization or allergy. What is an acceptable level of sensitization to foods in the population? Do sensitization and mild allergic reactions constitute harm under the Food Quality Protection Act's (28) standard of "a reasonable certainty of no harm"? There is no doubt that hospitalization from severe reactions would constitute such harm.

Conclusions

In 2000, the National Academy of Sciences (19) pointed to ways that the regulation of pest-protected plants can be improved and to a number of areas that need further research. The Starlink episode contributes a real-life example in which, in the absence of complete scientific information, the U.S. EPA attempted to limit the introduction of a new genetically modified organism by requiring that it only be used for animal feed. The inherent weaknesses in the identification of new allergens, described by the NRC report, largely contributed to the uncertainties about the risks of Starlink corn and the subsequent food crisis. In addition, there were fundamental flaws in risk management. In the absence of monitoring by the U.S. EPA or the FDA, perhaps it should not be so surprising that this variety was widely distributed in the food supply before the U.S. EPA (and Aventis) could take action to enforce the registration requirements. The credibility of this technology has been shaken by the uncertainty in the processes to assess and manage the risks of Starlink corn. Now industry is moving forward to attempt to construct better models for assessment of allergenic hazards of biotechnology, and agencies are developing more rigorous regulatory approaches.

There are several specific areas identified in this case study that suggest a need for changes in future approaches to the assessment and management of allergenicity of PIPs and other genetically modified food. First, and most obviously, there is a need for research to develop a fundamental basis for the assessment of risks of allergenicity. Such research needs to be relevant to the development of models for assessment of sensitization and triggering of allergic responses by proteins in food. Without a strong science basis for the development of such models, it will not be possible to identify levels of novel proteins that can be considered safe for introduction to the food supply. A closely related need is fundamental knowledge about the attributes of proteins that cause allergy. What are the structural characteristics of proteins

that render them allergenic to humans? Is it adequate to compare sequences and side groups, or are three-dimensional relationships more important?

From the standpoint of risk assessment of allergenicity, the approach of the U.S. EPA and the other agencies needs to be broadened to consider not only the potential for food allergy but also the potential for workplace and community allergic responses. Exposures to aeroallergens in work environments may be much greater than exposures via the food supply, depending on where the protein is expressed in the plant and on factors such as stability and digestibility of the protein (which are not relevant to inhalation exposure). Likewise, communities in proximity to farming or food-loading and processing operations may have significant exposures to these proteins.

From the standpoint of risk management, it is clear that, in the case of Starlink, the U.S. EPA trusted seed suppliers and growers to enforce restrictions on the planting of corn that were not maintained. Evidently, there was little governmental oversight to assure that the terms of the registration were obeyed. Any governmental system for regulation of PIPs and other genetically modified plants needs to employ risk-management strategies that are realistic and that can be monitored to assure they are followed. In addition, it appears that there may have been interbreeding between the Starlink corn and non-PIP corn, despite efforts to maintain buffer zones. If this proves to be the case, it calls into question the value of buffer zones, at least for corn.

It is also clear that there was no way of doing postmarket surveillance. Methods to assay food for novel proteins (and possibly DNA) and human sera for evidence of allergic sensitization need to be available before novel proteins go to market. Research is needed to develop and validate ELISA assays that can be used to confirm case reports of adverse allergic events.

It is becoming increasingly clear that, as predicted by the National Academy of Sciences report in 1987 (11), the hazards potentially posed by genetically modified organisms are not unique or impossible to deal with in the context of the experience gained with other organisms. At the same time, it should be recognized that genetically modified organisms might pose hazards to health that need to be assessed and managed. Identification of well-defined risks will reduce concerns over every genetically modified organism or over genetically modified organisms per se (an example could be a general shift from DNA to protein assays for exposure assessment). The Starlink episode is a consequence of an assessment science that has been outpaced by development of new

technology. Federal agencies and other stakeholders should become involved to guarantee that we have a broad approach to prevention of new food allergies. Implicit in this will be the need for an increase in research efforts in order to gain the fundamental knowledge that is needed for development of regulatory assessment methods. Recognition of the potential for risks should lead to better risk management that will include refinement of monitoring methods coupled with strong enforcement.

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