Case Studies in Benefits and Risks of Agricultural Biotechnology: Roundup Ready Soybeans and Bt Field Corn

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I. Introduction

Roundup Ready soybeans and Bt field corn have been rapidly adopted by U.S. farmers, yet their approval for commercialization remains under scrutiny. These case studies provide a description of the U.S. regulatory process governing agricultural biotechnology and traces the approval of Roundup Ready soybeans and Bt field corn, summarizing the information that was submitted to U.S. regulatory agencies by the registrants. This review applies to field corn only, and does not include Bt sweet corn or popcorn. Estimates of the impact that the adoption of these crops has had on U.S. agriculture are also provided. Potential risks that were evaluated during the regulatory review process include allergenicity, toxicity, pesticide resistance, out-crossing, non-target impacts and antibiotic resistance. Benefits include increased yields, reduced production costs and reduced pesticide use.

The regulatory structure for agricultural biotechnology has evolved over the past 25 years, as technology allowing for genetic modification of plants developed. The system continues to evolve as new and different applications of the technology emerge, and understanding of potential risks improves.

In reviewing the studies that were conducted on the safety of Roundup Ready soybeans, no indication of greater health or environmental risks were found compared to conventional varieties. The benefits of the introduction of Roundup Ready soybeans include savings of $216 million annually in weed control costs and 19 million fewer soybean herbicide applications per year.

The review of Bt field corn studies submitted to support approval shows that no indication of greater health or environmental risks were found, although conditional registrations were granted at the time of commercialization pending the submission of additional studies on non-target impacts and further development of insect resistance management programs. These re-registrations are currently under review by the Environmental Protection Agency. The primary benefit of the introduction of Bt field corn has been increased yields, by 66 million bushels in 1999. Growers have also achieved modest reductions in insecticide use, as only a small proportion of U.S. field corn acreage was sprayed for the target pest prior to the introduction of Bt varieties.

II. Risks

Concern has been raised about the risks associated with genetically engineered crop varieties. Potential human health risks include allergenicity, toxicity, and development of resistance to orally administered antibiotics. Environmental risks include potential for increased weediness of the crop plant, out-crossing of genetically modified plants with closely related wild plant species, non-target effects and the development of pesticide resistance. In response to these concerns, U.S. regulatory agencies assess the risks involved with the introductions of genetically modified plant varieties. The approval of crop varieties developed through biotechnology falls under the jurisdiction of three agencies: the U.S. Department of Agriculture (USDA), the Food and Drug Administration (FDA) and the Environmental Protection Agency (EPA). The risks of agricultural biotechnology are examined in the context of the regulatory framework that governs the introduction of genetically engineered crops in the U.S. The majority of studies that have been conducted to address the potential risks of these new crop varieties has been conducted by the developers of the technology in order to meet requirements of the regulatory agencies, which is standard practice.

A. U.S. Regulatory Framework

The U.S. regulatory framework for agricultural biotechnology has evolved over time as new technologies emerged that allowed the introduction of genetic material, beginning in the early 1970's. Over the past 25 years, policy has developed to address potential risks in a process open to public review and comment.

Initially, responsibility for oversight of the technology rested with the National Institutes of Health (NIH), but as applications of the technology changed, involvement of other agencies was deemed appropriate. As diverse products were presented for field-testing and commercialization (e.g., human insulin, ice minus bacterium, insect resistant tobacco, chymosin, rBST) involvement of the various agencies was required, and the system developed accordingly. Following is a brief overview of the development of regulations for agricultural biotechnology in the U.S.

Concerns about the potential dangers arising from new recombinant DNA (rDNA) techniques first arose in the early 1970's. In 1973, scientists gathered at an annual conference on nucleic acids, known as the Gordon Conference and heard descriptions of experiments where DNA molecules from diverse sources were joined. By the end of the conference, many attendees had voiced reservations about the ethical and moral problems as well as the safety issues that might arise from the technology. The conference attendees voted that a letter should be sent to the National Academy of Sciences pointing out that a problem had been raised meriting investigation (Goodfield). It was also decided to go public with the issue, by publishing their letter in Science on September 21,

1973 (Singer and Soll). The letter noted that although no hazards had yet been established, "prudence suggests that the potential hazards be seriously considered," and suggested that the Academies establish a study committee on the subject to recommend specific actions or guidelines as appropriate.

The National Academy of Sciences quickly convened a committee in 1974, publishing the recommendations in Science in July of that year (Berg, et al. 1974). The committee recommended that three types of experiments be deferred until the potential hazards were better evaluated or until adequate methods were developed for preventing the spread of biologically active recombinant DNA molecules: constructing replicating plasmids that would introduce either antibiotic resistance or bacterial toxins into bacterial strains; linking DNA from likely cancer-causing viruses to bacterial plasmids; the linking of fragments of animal DNA to bacterial plasmid DNA or bacteriophage DNA. The committee suggested that the director of NIH establish an advisory committee to develop an experimental program to evaluate the hazards, develop procedures that would minimize the spread of such molecules within populations and devise guidelines to be followed by investigators. They also called for an international meeting of scientists to further discuss appropriate ways to deal with the potential biohazards of recombinant DNA molecules.

The Asilomar Conference was held in 1975, convening nearly 140 international scientists to "review scientific progress in research on recombinant DNA molecules and to discuss appropriate ways to deal with the potential biohazards involved" (Berg, et al. 1975). The recommendations of the conference consolidated and extended those of the National Academy of Sciences Committee.

The Recombinant Molecules Advisory Committee (RAC) of the NIH began meeting as soon as the Asilomar Conference ended, working on research safety issues of experimental facilities and personnel, as well as of the proposed experiments themselves. In February 1976, the director of NIH called a public hearing in response to increased public interest in the subject. Four months later, in June 1976, NIH published its final guidelines for laboratories conducting recombinant experiments under federal grants (Goodfield).

As a standing committee, the RAC meets periodically to address and incorporate emerging scientific understanding of the potential risks involved with rDNA technologies. By 1983, experience with rDNA had allayed many fears, and NIH guidelines had been successively weakened to allow experiments that had been delayed awaiting better understanding of the associated risks. NIH had become comfortable with the vast majority of ongoing basic and biomedical research (Thompson). Risk assessment work helped assure the scientific community and the public that many rDNA experiments were not as hazardous as originally believed (Korwek).

Although the NIH Guidelines govern only federally funded research, private industry and trade associations generally abide by the Guidelines as well. Through

institutional biosafety committees, private industry reviews risk and ethical concerns of prospective research areas, referring any questions to the RAC for advice and consultation. It is believed that individuals and institutions that are not required to follow the NIH Guidelines do so for legal liability concerns. A 1987 General Accounting Office report found that private companies appeared to follow the Guidelines more closely than public sector organizations (Korwek).

The landscape of risk issues changed in the early 1980's as genetic engineering was to move out of the laboratory and into agricultural fields with the development of "ice minus," a genetically altered bacterium intended for use on a variety of crops to reduce the risk of freezing. The regulation of a product that was to be purposely introduced into the environment presented quite a different set of issues than those involved with laboratory experimentation, the risks of which were controlled primarily by containing engineered materials and insuring against introduction into the environment.

Originally proposed in 1983, field testing of "ice minus" was delayed through a series of legal challenges for four years. During this time, the authority of NIH over field tests was questioned, and EPA, USDA and FDA were proposed as the appropriate bodies for regulating in this area. The lack of coordination and uncertainty about oversight of biotechnology led to the formation of an interagency working group under the White House Cabinet Council on Natural Resources and the Environment. The working group was composed of approximately 13 member agencies, as an interagency effort to review regulatory requirements for conventional technologies, to clarify regulatory requirements for new products and to determine whether current regulatory requirements were adequate. Initial results of the working group were published for public comment in the Federal Register in 1984 (OSTP 1984). The Office of Science and Technology Policy (OSTP) published its final notice of how each agency would regulate biotechnology applications in 1986, in the policy that would become commonly known as the "Coordinated Framework" (OSTP 1986). In this Notice, existing laws were deemed adequate to oversee modern biotechnology applications. The notice also set forth which regulatory bodies were designated as the lead agency where the possibility of duplication of oversight existed (Korwek). USDA is the lead agency for plants grown to produce food or feed crops, while the food or feed itself is subject to regulation by FDA. EPA would primarily handle pesticide microorganisms. Notably, the initial policy of EPA addressed microbial pesticides, but did not address the regulation of pesticidal plants, which had not yet been developed at that point.

EPA, USDA and FDA each issued statements outlining their regulatory policy, in the Coordinated Framework. A common theme in the policies of all three agencies is the concept of product- not process-based risk assessment, based on the conclusion that the risks associated with the introduction of rDNA-engineered organisms are the same as those associated with introductions of unmodified organisms and those modified by other methods. This concept was supported by three reports by the National Research Council (NRC).

The first report was published in 1987, entitled Introduction of Recombinant DNA-Engineered Organisms into the Environment: Key Issues, which concluded that the risks associated with the introduction of genetically engineered organisms were the same as those associated with introductions of unmodified organisms and those modified by other methods. In 1989, the NRC issued Field Testing Genetically Modified Organisms: Framework for Decisions, which more specifically addressed the scientific foundation for regulatory decisions governing the release of genetically engineered microorganisms and plants into the environment. The 1989 report further supported the concept of the product not process-based standard for oversight put forth in the 1987 study. In 2000, the NRC released a report entitled Genetically Modified Pest-Protected Plants: Science and Regulation, the purpose of which was primarily to evaluate the EPA's regulatory system for pesticidal plants. In the 2000 report, the committee was critical of EPA's policy of exemptions for plant varieties produced using particular methods.

The scope of regulation was the subject of a review prepared by the White House Council on Competitiveness, published in 1990 for public comment in the Federal Register. This document excluded from regulation organisms developed by traditional techniques, though the document did not propose any rules. The Council later published four principles of regulatory review for biotechnology and a report on national biotechnology policy (President's Council on Competitivness). These principles, along with the final scope document, published in 1992 (OSTP 1992), articulated a risk-based approach to regulation.

1. USDA

The USDA Animal and Plant Health Inspection Service (APHIS) is responsible for protecting U.S. agriculture from pests and diseases. Under the Federal Plant Pest Act, USDA retains the authority to regulate plant pests and other articles to prevent direct or indirect injury, disease, or damage to plants, plant products, and crops. In 1987, USDA published regulations that finalized the rule that was proposed under the Coordinated Framework (USDA APHIS 1987). The requirements extended regulations imposed by APHIS for non-genetically engineered organisms or products that are plant pests or could harbor plant pests. APHIS promulgated these new regulations because it deemed that the existing regulations did not provide any way to determine whether or not a genetically engineered organism or product would fall under existing regulations of plant pests. The rule specifically notes that APHIS is not treating genetically engineered organisms and products differently than non-genetically engineered organisms. These regulations were amended in 1993 and 1997 (USDA APHIS 1993a; USDA APHIS 1997).

The regulations provide the rationale for determining whether a genetically engineered organism or product would be considered a "regulated article," i.e., one with plant pest characteristics, and also calls for additional data for making a determination on the plant pest status of certain genetically engineered organisms or products. A genetically engineered organism is deemed a regulated article either if the donor

organism, recipient organism vector or vector agent used in engineering the organism is listed in the regulation and is also a plant pest, is unclassified, or if APHIS has reason to believe that the genetically engineered organism presents a plant pest risk. This criterion for determining whether a particular modified plant is subject to regulation by APHIS was criticized in the recent National Research Council report, which noted that some pest-protected plant varieties did not fall under its scope given the definition of regulated article (NRC 2000).

APHIS is responsible for approving introductions of genetically modified crops at two stages: for field trials, and for full market release. Prior to conducting field trials, it is necessary to either obtain a permit or to notify APHIS. The notification option was established in 1993 for certain regulated articles with which the department is familiar, provided that the introduction is conducted in accordance with established requirements and standards (USDA APHIS 1993a).

APHIS regulations also provide for a petition process for the determination of nonregulated status, which allows the unregulated movement and release of the product. In the petition for nonregulated status, applicants must "describe known and potential differences from the unmodified recipient organism that would substantiate that the regulated article is unlikely to pose a greater plant pest risk than the unmodified organism from which it was derived" (USDA APHIS 1993a).

Environmental Assessments are prepared for field trials, and for petitions for nonregulated status. These assessments detail the nature of the genetic modification and assess the potential for environmental impacts from the introduction of the crop varieties into the environment. When a product is approved for full release, a Determination of Nonregulated Status is published in the Federal Register.

Lack of plant pest risk may be concluded when there is evidence that the plant under consideration: (1) exhibits no plant pathogenic properties; (2) is no more likely to become a weed than its non-engineered parental varieties; (3) is unlikely to increase the weediness potential for any other cultivated plant or native wild species with which the organism can interbreed; (4) does not cause damage to processed agricultural commodities; and (5) is unlikely to harm other organisms, such as bees, that are beneficial to agriculture.

2. EPA

The Environmental Protection Agency (EPA) assesses the safety of pesticides, both chemical and those that are produced biologically. Under the authority of the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), EPA regulates the distribution, sale, use and testing of plants and microbes producing pesticidal substances. Under the Federal Food, Drug and Cosmetics Act (FFDCA), EPA sets tolerance limits, or maximum allowable residue concentrations, for substances used as pesticides on and in

food and feed, or establishes an exemption from the requirement of a tolerance. EPA also establishes tolerances for residues of herbicides used on novel herbicide-tolerant crops.

The goal of FIFRA is to register pesticides that do not have unreasonable adverse effects on human health or the environment and have benefits outweighing risks. Unreasonable adverse effects on the environment are defined as any unreasonable risk to "man or the environment, taking into account the economic, social, and environmental costs and benefits of the use of any pesticide" (Korwek).

Any substance that is considered a pesticide under FIFRA is automatically subject to regulation under FFDCA if used on a food or feed crop (Nelson and Abramson). Until recently, EPA's decisionmaking under FFDCA also involved a balancing of risks and benefits, however, only dietary risks to humans and other animals were considered, as opposed to FIFRA which also takes into account environmental risks (US EPA 1994). However, since passage of the Food Quality Protection Act in 1996, Congress has required EPA to apply a safety-only standard when examining the potential dietary risks associated with pesticide residues that may be found in food (Nelson and Abramson).

Early policy statements of EPA were focused on the regulation of genetically modified microbial pesticides. A 1984 statement of interim policy required notification prior to small scale field tests involving certain microbial pesticides, including those that had been genetically altered, in order to determine whether an experimental use permit (EUP) would be required for testing (US EPA 1984). In 1986, as part of the Coordinated Framework, EPA published its statement of policy pertaining to regulating microbial pesticides under FIFRA, which sought to define which microbial products would be subject to review under FIFRA as well as the nature of the review (OSTP 1986). In a 1989 Notice, EPA requested comments on the regulatory approach to microbial pesticides articulated in the 1986 policy statement (US EPA 1989). A proposed rule was published by EPA in 1993, based upon the 1984 interim policy and the 1986 proposed policy, addressing the requirements for small scale field testing of microbial pesticides, as regards notification and EUP's (US EPA 1993).

It was not until 1994 that the agency began to publish policy applicable to genetically modified organisms other than microbial pesticides and products. That year, EPA published a proposed policy for "plant pesticides" to be regulated under FIFRA and FFDCA. The 1994 proposed policy annunciated the agency's intent to regulate the pesticidal substances in plants, but not the plants themselves, leaving the regulation of the plants to USDA. This stance followed from an earlier policy by EPA to exempt from regulation under FIFRA all biological control agents, except for certain microorganisms, which has been interpreted to include plants (US EPA 1994). Since the time that the 1994 proposed policy was issued, EPA has proposed replacing the term "plant pesticide" with "plant-incorporated protectant."

Several exemptions were proposed in the 1994 statement. First, plant pesticides derived through conventional breeding methods would be granted a generic exemption

from registration under FIFRA. Further, EPA proposed to exempt from regulation under FIFRA plant pesticides that are derived from sexually compatible plants. Viral coat proteins were also proposed to be exempt under FIFRA. Three categories of exemptions from tolerance setting under FFDCA were also proposed: plant pesticides that would not result in new dietary exposures, nucleic acids in plants, and coat proteins from plant viruses. With these exemptions, the agency intended to regulate those plant-pesticides that have the greatest potential for adverse effects, on both the environment or on health (US EPA 1994).

A recent report by the National Academy of Sciences addressed the issue of the exemptions proposed by EPA. Though the committee agreed that conventionally bred plants should be exempt for practical reasons based on historical safe use and benefits of these crops, the committee questioned the scientific basis used by EPA for this exemption. Regarding the exemption for plant pesticides derived from sexually compatible plants, the committee questioned the categorical nature of the exemption, while noting that exemptions for certain sexually compatible transgenic plant pesticides would be appropriate. The committee agreed that viral coat proteins should be exempt from regulation under FFDCA, but questioned the exemption under FIFRA due to concerns about potential outcrossing with weedy relatives (NRC).

The 1994 proposed policy also describes the risk issues with which the regulations are concerned. The following environmental risk issues are considered for both field testing and sale or distribution of a plant pesticide: increasing the ability of the modified plant to survive outside of cultivation through the introduction of a specific trait; gene capture and expression of the introduced trait by a wild or weedy relative; potential for a trait conferring a selective advantage to a plant in a natural plant community with the reuslt of increasing the “weediness” of that species; environmental fate of the pesticidal substance, the dosage to soils after plant senescence and incorporation into the soil, rate of degradation or dissipation and transport in the environment. Also whether or not the pesticidal substance is either exuded or volatilized from the plant during the growing season, resulting in a continuous application to the environment (US EPA 1994).

Under FFDCA, EPA maintains jurisdiction over food safety issues related to the plant pesticide. Food safety issues related to compositional changes in the plant itself areunder FDA jurisdiction. Environmental issues related to the plant itself are regulated by USDA APHIS, as mentioned above.

3. FDA

FDA regulates foods and food ingredients, including animal feed and feed additives, under the FFDCA. The agency’s authority to regulate the safety of food is generally exercised under two sections of the Act. Section 402(a)(1) applies to unintended occurrences of unsafe levels of toxicants in food. This section is the agency’s primary legal tool for regulating the safety of whole foods, placing liability for food safety

on the producer of a new food, and it is under this section that new plant varieties, including those produced using conventional techniques, have historically been regulated. Under this section, the agency retains the authority to remove a food from commercialization if it is found to be unsafe. However, under this section, there is no requirement for safety testing prior to commercialization. Section 409 of the act applies to food additives, or intentional changes in the composition of foods. Under this section, premarket approval is required unless the food additive is generally recognized as safe (GRAS), or is a pesticide and therefore regulated by the EPA. The GRAS exception allows many ingredients derived from natural sources (e.g., salt, pepper, spices) and some chemical additives (some sweeteners, preservatives, artificial flavors) to be marketed without having been formally reviewed by FDA (US FDA 1992).

In its 1986 statement, as part of the Coordinated Framework, FDA announced its intention to apply the existing regulatory framework to genetically engineered plant varieties. In that statement, FDA clearly states its intention to base its regulation of food on rational and scientific evaluation of the product, not on the process used to develop the product (OSTP 1986).

Further refinements to FDA policy were made in 1992 as the agency issued its policy statement establishing the regulatory framework under which FDA currently operates with regard to foods developed using biotechnology (US FDA 1992). Under the 1992 policy, regulation of genetically engineered varieties under the food additive provisions of FFDCA which would require premarket review are interpreted to apply to the transferred genetic material and the intended expression product. The introduced genetic material itself is considered to be GRAS, as nucleic acids are present in the cells of every living organism. Expression products, such as proteins, carbohydrates, fat or oil, would only require premarket review if they differ significantly in structure, function or composition from a substance found currently in food, or sufficient safety issues are raised.

Several scientific issues are highlighted in the 1992 statement, including unintended effects, known toxicants, nutrients, new substances, allergenicity, and antibiotic resistance selectable markers. These issues are the focus of FDA regulation of new plant varieties.

FDA has been particularly attuned to the potential of new plant varieties to cause allergies. The agency's principal concern is the possibility that an allergy-causing protein would be transferred from one food plant to another, making the recipient plant cause an allergic response in those allergic to the donor plant. In the case where a protein is derived from a commonly allergenic source, it is possible to test the new variety for allergenic responses in individuals known to be sensitive to the donor plant. For proteins that are derived from non-food sources, testing for potential allergenicity is less straightforward.

In April 1994, FDA, EPA and USDA hosted a scientific conference on allergenicity in transgenic food crops. Attendees concluded that methods are available to assess allergenic potential for proteins that are derived from sources to which consumers have reacted and for which serum is available, but it may be useful to establish a serum bank. There are no direct methods to assess potential allergenicity of proteins from sources that are not known to produce food allergy. Some assurance can be provided to minimize the possibility that a new protein will cause an allergic reaction by evaluating its similarity with characteristics of known food allergens. However, this is an area where more research has been called for. The National Academy of Sciences recommended that priority be given to developing improved methods for identifying potential allergens (NRC 2000).

FDA is also concerned with the use of antibiotic resistance marker genes in transgenic plants and the risk of reducing the effectiveness of antibiotics in humans and animals. The kanamycin resistance marker gene is commonly used in transgenic plants. Calgene, the developer of the Flavr Savr tomato, the first transgenic crop to be approved by FDA, requested that FDA subject the kanamycin resistance gene to evaluation under food additive regulations. At the time, FDA convened a Food Advisory Committee to consider Calgene's petition. The committee considered both direct risks of allergenicity and toxicity and the effects on the efficacy of antibiotics.

The 1992 policy statement includes a section on guidance to the industry for foods derived from new plant varieties, which describes scientific considerations for the evaluation of the safety and nutritional aspects of new plant varieties. The guidance section of the statement includes decision trees to assist developers in determining whether their product would be subject to regulation as a food additive or if consultation with FDA is necessary to determine the regulatory status of the product. Informal consultation with the agency has been standard practice for the food industry, and FDA expects that developers of genetically engineered varieties would continue this practice (US FDA 1992).

One controversial aspect of the FDA policy is that no premarket review has been required for these crops. Consultations have been technically voluntary, though the Agency knows of no product that has been commercialized without prior consultation with the Agency. However, consultations with the Agency recently became mandatory (US FDA 2000).

The most controversial aspect of FDA's policy has been the decision that foods developed using rDNA technology would not require labeling. This decision was based on the judgement that these products do not differ in any significant way from their conventional counterparts solely due to the process through which they were developed. It should be noted that labeling is required for genetically engineered foods that contain genetic material from foods that are commonly allergenic, unless it can be demonstrated that the allergenic property has not been transferred to the new plant variety. Further,

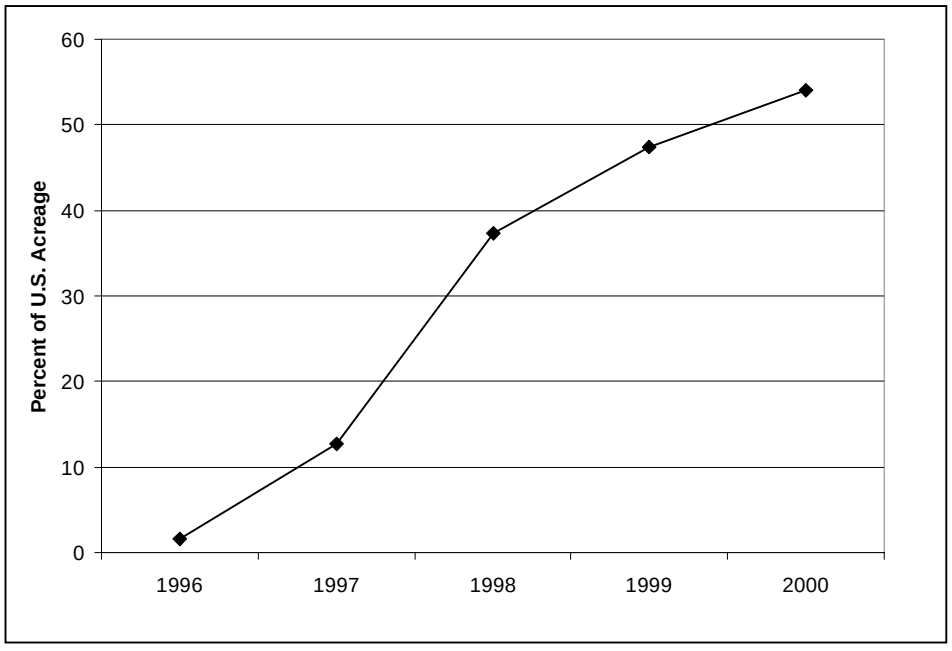
plant varieties that have altered nutritional characteristics, such as modified oil content, would also require labeling (US FDA 1993).

B. Roundup Ready Soybeans

The development of crops tolerant to the herbicide glyphosate (Roundup) began in the early 1980s. The first generation of glyphosate tolerant soybeans was grown in a greenhouse during the winter of 1990-1991, the seeds of which were then planted in field tests during the summer of 1991 (Padgette, et al. 1996a). Approvals for commercialization of glyphosate tolerant soybeans were granted by FDA and USDA in 1994 and by EPA in 1995. Glyphosate tolerant soybeans, commonly known as "Roundup Ready" soybeans, were first made available for planting by U.S. farmers in 1996.

Glyphosate tolerant soybean varieties have been widely adopted by U.S. growers. Figure 1 shows adoption of glyphosate tolerant soybeans since 1996 in the U.S. By 2000, growers planted 54% of U.S. soybean acreage to glyphosate tolerant soybeans (USDA NASS 2000a).

Figure 1. Glyphosate Tolerant Soybean Adoption



Sources: Marshall, USDA NASS 2000a

Glyphosate controls weeds by inhibiting the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), which catalyzes the synthesis of amino acids essential for survival of plants and bacteria. EPSPS is present in plants, bacteria and fungi, but not

animals, as animals do not make their own aromatic amino acids but rather receive them from plant, microbial or animal-derived foods.

A number of bacterial species have been shown to exhibit tolerance to glyphosate. A glyphosate tolerant EPSPS from the soil bacterium *Agrobacterium* sp. strain CP4 was isolated and introduced into the genome of a soybean cultivar using the particle acceleration method. DNA is coated onto microscopic gold particles, which are then accelerated and penetrate target plant cells. Resulting cells are then incubated to produce shoots, which will eventually grow into mature plants. Successfully transformed plants are selected that exhibit unaltered agronomic traits from the parent line.

Soybean is the second largest crop in the U.S. after corn, planted on 74.5 million acres in 2000 (USDA NASS 2000a). Acreage planted to soybean has expanded in recent years due to several factors. Improved yields through variety improvements, adoption of moisture-saving no-till practices, strong soybean prices relative to other crops, and elimination of acreage reduction programs are all factors that have contributed to expanded plantings. Total soybean crop value in 1999 was $13 billion (USDA ERS).

Soybean acreage is centered in the Midwestern states, though 30 states have significant acreage planted to soybeans each year. Illinois and Iowa each plant over 10 million acres of soybeans. Other major soybean states include Minnesota, Indiana, Missouri and Ohio.

The U.S. is the largest producer of soybeans in the world, growing nearly half of the total world soybean crop. Other major producing countries include Brazil, China and Argentina. The U.S. exports approximately one-third of its soybean production, primarily to Asia and Europe, which together account for over 70% of total exports. Competition in export markets comes from Brazil and Argentina, as China is a net importer of soybeans (USDA NASS 2000b).

1. USDA

APHIS received a petition from Monsanto on September 15, 1993, seeking a determination from APHIS that glyphosate-tolerant soybean (GTS) line 40-3-2 and its progeny do not present a plant pest risk and are therefore not regulated articles (Re, et al. 1993). On December 6, 1993, APHIS announced receipt of the Monsanto petition in the Federal Register, stating that the petition was available for public review (USDA APHIS 1993b).

APHIS received 33 comments on the Monsanto petition. With one exception, the comments were favorable to the petition. The one unfavorable comment stated that USDA should not approve the Monsanto petition or any other petition until the federal government has revised its oversight program for transgenic crops at the commercialization stage, including establishment of standardized assessment and data

collection schemes for consideration of risks of transgenic crops to ecosystems in the U.S. and world-wide, with particular attention to centers of diversity for food and fiber crops. The commenter also expressed the view that development of herbicide-tolerant crops should not be encouraged because they increase farmers' dependence on chemical herbicides (USDA APHIS 1994a).

The functional Roundup Ready gene contained in GTS line 40-3-2 is contained on a single insert of DNA comprised of the enhanced 35S promoter derived from cauliflower mosaic virus, the chloroplast transit peptide coding sequence from Petunia hybrida fused to the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) gene derived from Agrobacterium sp. strain CP4, and the nopaline synthase 3' terminator from A. tumefaciens.

GTS line 40-3-2 has been considered a "regulated article" because it contains components from organisms that are known plant pathogens, i.e., the bacterium Agrobacterium tumefaciens and cauliflower mosaic virus. Field testing of GTS line 40-3-2 had been conducted with APHIS approval since 1991. Monsanto submitted its petition after completion of field tests of GTS line 40-3-2 under 9 APHIS permits. These permitted field tests took place at approximately 54 sites in 19 states and Puerto Rico. Additional trials were conducted in the U.S. and Puerto Rico under permit and notification during the 1993 growing season. All field trials were performed under conditions of physical and reproductive confinement.

The Monsanto petition describes the genetically engineered soybean plants and provides information relevant to determining whether glyphosate tolerant soybean plants are more likely than conventional varieties to become a plant pest. The petition addresses potential environmental consequences of unregulated release of glyphosate tolerant soybean varieties, including the development of glyphosate tolerant weeds, enhanced weediness, effects on nontarget organisms, impacts of human and animal exposure, indirect effects on other agricultural products and the potential for outcrossing. Reports from field trials are included with observations of yields, plant growth, outcrossing, survival and gene expression gathered during field tests of glyphosate tolerant varieties compared to conventional varieties. Examples of the monitoring forms used by investigators who conducted the experiments are also included in the petition. In addition, letters from six land grant university weed scientists are included addressing the potential for development of weed resistance to glyphosate, weed population shifts and the overwintering of glyphosate tolerant soybeans, which were issues of concern to USDA.

APHIS granted the petition in May 1994, issuing a Finding of No Significant Impact. This conclusion was based upon the nature of the genetic modification, the fact that soybean has no weedy relatives with which it can interbreed in the U.S. and its territories and the conclusion that this modification will not increase the weediness of the soybeans or negatively effect any nontarget organisms, including beneficials (USDA APHIS 1994b).

Weediness

Soybean (*Glycine max*) possesses few of the characteristics of plants that are notably successful weeds. *Glycine max* cv. 5403, the cultivar which was genetically modified, is not considered to be a weed, and glyphosate tolerance is not expected to confer any additional weedy characteristics. Standard texts and lists of weeds give no indication that cultivated soybean is regarded as a weed anywhere (USDA APHIS 1994a). Overwintering of soybeans is rare due a lack of innate dormancy. A lack of dormancy is selected for in commercial soybean seeds, so soybean seeds germinate quickly. Any seed that might remain in a field after harvest is likely to germinate, emerge and be killed by frost or field preparation for the following crop. Very few volunteers were observed in field testing. The number of seeds produced, germination characteristics, final stands, overwintering capability and disease or insect susceptibility were all found to be similar for the tested glyphosate tolerant line compared to conventional varieties. These findings were based on yield data and observations of germination, stand counts and disease or insect susceptibility. Further, increased weediness of the glyphosate tolerant soybean plant compared to conventional varieties would have to be due to selection pressure in association with glyphosate use. This was judged not to be an issue since glyphosate is not applied to the soybean for control of the soybean itself, but rather for controlling weeds in the field (USDA APHIS 1994a).

Outcrossing

The genus Glycine is divided into two subgenera, Glycine and Soja. The first consists of 12 wild perennial species that are primarily distributed in Australia, South Pacific Islands, Philippines, and Taiwan. The subgenus Soja consists of three annual species from Asia, G. max, G. soja and G. gracilis. The first species is the cultivated soybean, the second species is the wild form of the soybean, and the third species is referred to as the "weedy" form of the soybean.

Cultivated soybean is sexually compatible only with members of the genus Glycine. Cultivated soybean is the only member of the genus Glycine that grows in the US and its territories and is sexually compatible with cultivated soybean, with the exception of specialized research collections. However, some members of the wild perennial species of subgenus Glycine may be found in U.S. territories in the Pacific. There are no known reports of successful natural hybridization between the cultivated soybean and the wild perennial species.

The wild annual species, G. soja is found in China, Taiwan, Japan, Korea, and the former USSR. Natural hybridizations between G. soja and cultivated soybean occurs. G. soja is not native to North America and occurs only in research plots. There are no reports of its escape or dispersal from research plots. G. soja has never been found as a weed or naturalized in the U.S. Thus, the possibility of gene transfer is very low within the U.S.

Even if non-agricultural land containing any wild Glycine populations were near sites of commercial soybean production, it is highly unlikely that pollen from GTS line 40-3-2 would fertilize the wild relative, because soybeans are almost completely self pollinated. The anthers mature in the bud and shed their pollen directly onto the stigma of the same flower, thus ensuring a high degree of self-pollination. Cross-pollination is generally very low and various studies have shown it to be from 0.03 to 3.62%. Honeybees are responsible for the occasional cross-pollination.

The limited potential for cross-pollination is evident in certified seed regulations for Foundation seeds, the most sringent category in the Certified Seed Regulations, which permit zero distance between different soybean cultivars in the field.

Non-Target Effects

Glyphosate tolerant soybeans were judged to have no detrimental effects on non-target organisms. EPSPS enzymes are present in plants and microorganisms and are therefore normally found in food and feed. No effects on non-target organisms were expected. The glyphosate tolerant EPSPS that was introduced into soybeans is not known to have any toxic properties. Field observations revealed no negative effects on non-target organisms including insects, birds or other species that frequent soybean fields (USDA APHIS 1994a).

Weed resistance

Although the development of herbicide resistant weeds is not specifically considered by USDA in the approval process, Monsanto's petition to USDA provided information addressing this possibility. Glyphosate is considered to be an herbicide with a low risk for the development of weed resistance. Major factors which can contribute to the development of resistant weeds include: a single target site and a specific mode of action, broad spectrum of activity, long residual activity, and frequent applications without rotation to other herbicides or cultural control practices. Glyphosate essentially has no residual activity in the soil and is relatively quickly broken down by microorganisms in the soil. Also, there is no other herbicide on the market today that has the same mode of action as glyphosate. Glyphosate has been widely used for over 20 years, as a pre-plant burndown, directed, spot, or post-harvest treatment. However, some have questioned the impression of "invincibility" of glyphosate to the development of resistance (Gressel). Resistant weed populations have been reported in Malaysia and Australia (Doll; Sindel).

Plant pest risk

APHIS also assessed the possibility that glyphosate tolerant soybeans would pose a plant pest risk due to the presence of pathogen-derived sequences. Neither of the gene sequences from *Agrobacterium tumefaciens* nor from the cauliflower mosaic virus cause any plant or animal disease, is the source of pathogenicity in its host or encodes any

polypeptide. No crown gall, the disease caused by *A. tumefaciens*, nor cauliflower mosaic virus disease were observed in any glyphosate tolerant soybean plants during greenhouse or field studies.

Yields

Further information was submitted by Monsanto on November 19, 1993, to address a slight yield reduction observed at three of seven sites in initial yield trials.

2. EPA

Crops that have been genetically modified to be herbicide tolerant do not face regulation under FIFRA, as the plants contain no pesticidal substance. EPA must grant any changes in tolerances for residues that might be needed to accommodate altered use patterns for in-season applications of the herbicide. Further, EPA must approve the modification of the label for the herbicide to allow for in-season use of the herbicide over the growing crops, which would not have been allowed previously.

In April 1996, EPA established new tolerances and feed additive regulations for the residues of glyphosate on several commodities for several end uses, in response to a number of petitions submitted by Monsanto. The revised tolerances were based on data submitted from several toxicological studies, as summarized in Table 1. In addition to those studies listed in Table 1, several acute toxicology studies were submitted that placed technical grade glyphosate in Toxicity Categories III and IV. All mutagenicity tests were negative. The carcinogenic potential of glyphosate has been judged to belong in Group E, evidence of noncarcinogenicity for humans, based on lack of convincing carcinogenicity evidence in adequate studies in two animal species (US EPA 1996a). Revised tolerances for glyphosate are provided in Table 2. EPA approved a change in the label for Roundup to allow use of Roundup over the top of growing soybean plants in 1995. Since this change did not affect the registration of Roundup, this approval was not published in the Federal Register (Korwek).

Table 1. Glyphosate Mammalian Toxicology Test Results Submitted to Support Revised Glyphosate Tolerances

Subject Animal	Type of Study	Dosages	Results
Dogs	1-year Feeding	0, 20, 100 and 500 mg/kg/day	NOEL 500 mg/kg/day
Mice	2-year Carcinogenicity	0, 150, 750, 4500 mg/kg/day	No carcinogenic effects at 4500 mg/kg/day
Rats	Chronic Feeding/ Carcinogenicity	0, 3, 10 and 31 mg/kg/day (males) 0, 3, 11, 34 mg/kg/day (females)	No carcinogenic effects at any dose level; Systemic NOEL of 31 mg/kg/day (males); Systemic NOEL of 34 mg/kg/day (females)
Rats	Chronic Feeding/ Carcinogenicity	0, 89, 362 and 940 mg/kg/day (males) 0, 113, 457 and 1183 mg/kg/day (females)	No carcinogenic effects at any dose level; Systemic NOEL of 362 mg/kg/day (males) based on increased incidence of cataracts and lens abnormalities, decreased urinary pH, increased liver weight and increased liver weight/brain ratio at 940 mg/kg/day (males); Systemic NOEL of 457 mg/kg/day (females) based on decreased body weight gain 1183 mg/kg/day.
Rats	Developmental	0, 300, 1000, 3500 mg/kg/day	Developmental NOEL of 1000 mg/kg/day based on an increase in number of litters and fetuses with unossified sternebrae, and decrease in fetal body weight at 3500 mg/kg/day; Maternal NOEL of 1000 mg/kg/day based on decrease in body weight gain, diarrhea, soft stools, breathing rattles, inactivity, red matter in the region of nose, mouth, forelimbs, or dorsal head, and deaths at 3500 mg/kg/day.

Rabbits	Developmental	0, 75, 175, 350 mg/kg/day	Developmental NOEL of 350 mg/kg/day; Maternal NOEL of 175 mg/kg/day based on increased incidence of soft stool, diarrhea, nasal discharge and deaths at 350 mg/kg/day.
Rats	Multigenerational reproduction	0, 3, 10, 30 mg/kg/day	Developmental NOEL of 10 mg/kg/day based on increased incidence of focal tubular dilation of the kidney of F3b pups.
Rats	Two generation reproduction	0, 100, 500, 1500 mg/kg/day	Developmental NOEL of 500 mg/kg/day based on decreased pup body weight and body weight gain on lactation days 14 and 21 at 1500 mg/kg/day; Systemic NOEL of 500 mg/kg/day based on soft stools in F0 and F1 males and females at 1500 mg/kg/day; Reproductive NOEL of 1500 mg/kg/day.

NOEL-No observable effects level

Table 2. Glyphosate Tolerances in Soybeans (ppm)

	Revised Tolerance
Soybeans	20
Soybeans,grain	20
Soybeans, aspirated grain fractions	50
Soybeans, forage	100
Soybeans, hay	200

Sources: EPA 1996a

3. FDA

Monsanto began the consultation process with FDA in June 1993. In accordance with the consultation guidelines, data was provided that described the crop that was being transformed, the introduced genetic material, the identity and function of the expression product, comparison of composition of genetically modified and conventional soybeans, as well as data and information addressing potential allergenicity and toxicity issues.

The safety evaluation can be broken down into two categories, unintended effects and intended effects, in accordance with the statutory structure, which regulates these effects differently, requiring premarket review only for intended effects under section 409.

Unintended effects

In order to address the possibility of the genetic modification having unintended effects on the crop, studies were performed to assess the composition of the genetically modified soybeans compared to conventional soybeans. In addition, wholesomeness studies were performed to evaluate any differences in feeding characteristics of genetically modified soybean feed and conventional feed.

In the compositional analysis, evaluations were performed on seed, toasted meal, defatted meal (flour), protein isolate, protein concentrate, crude lecithin, and refined, bleached, deodorized oil. Differences in seed composition were seen to be an indication that differences in other products would be found. Other products were chosen as they represent the various uses of soybeans. Toasted meal is widely used in animal feed. Defatted meal, protein isolate and protein concentrate are commonly used in food, as are lecithin and soybean oil. For seeds, the parameters compared were: protein, fat, fiber, ash, carbohydrate, amino acids, fatty acids, soybean seed proteins, trypsin inhibitor, lectin and isoflavones. The aromatic amino acids were of particular importance, since the CP4 EPSPS protein catalyzes a step in the aromatic amino acid pathway.

Significant differences in fat, ash and carbohydrate content were observed in one study, while no significant differences in these parameters were observed in similar studies conducted the next year. No significant differences were observed for the aromatic amino acids. The lack of change in the levels of aromatic amino acids confirmed that EPSPS is not the rate-limiting step in the aromatic acid biosynthesis. Protein, fat, fiber, ash and carbohydrate content were measured for defatted toasted meal, defatted non-toasted meal, protein isolate and protein concentrate, and no significant differences were found for these values between genetically modified soybeans and conventional soybeans. Antinutrient content (trypsin inhibitor and urease, phytate, stachyose, raffinose, lectins, isoflavones) were measured in toasted meal and, apart from lectin concentrations which were below detection limits, no significant differences were found. Fatty acid composition was measured for soybean oil and no significant differences were found. The composition of crude lecithin produced from GTS line 40-3—2 was compared and found to be comparable and equivalent to lecithin produced from conventional soybeans.

Animal feeding studies were performed using rats, broiler chickens, dairy cattle, catfish and bobwhite quail. Two separate rat studies were performed, using processed and unprocessed meal. The study using processed meal was intended to address mammalian health issues, while the unprocessed meal was intended to address risks to wild animals that might feed on unharvested beans in the field. Broilers were included due to the prevalent use of processed soybeans in broiler operations. Similarly, dairy cattle were fed raw soybeans, due to the widespread use of soybeans in cattle feed. Catfish were fed processed meal as this comprises a great portion of feed used in aquaculture. Finally, bobwhite quail were fed unprocessed soybeans in order to address potential risks to birds that might feed on soybeans in the field. These studies were not designed as toxicology tests, but rather were undertaken to determine whether there were any differences in wholesomeness, or the ability to support growth and well-being. Table 3 summarizes the setup of the animal studies that were performed by Monsanto. It was concluded that no material differences were found in the wholesomeness of soybean products in any of the animal studies.

Intended Effects

In the evaluation of intended effects, several aspects of the genetically modified crop are considered: expression level of introduced protein; similarity of introduced protein to those already common in food and feed; allergenic potential; toxicity; prevalence of protein in food and feed; and changes in carbohydrate, fat or oil composition, structure or levels.

The expression level of CP4 EPSPS in soybean seed and processed soybean products was evaluated. In whole seed, the concentration of CP4 EPSPS was found to be 0.3 μg/mg fresh weight. Concentrations in toasted meal, defatted meal, protein isolate and protein concentrate were measured and found to be less than 0.1% of total protein. No enzymatic activity was found in any of the processing fractions.

The introduced protein, CP4 EPSPS was found in to be similar to EPSPS's already commonly present in food due to similarity in the reaction catalyzed, amino acid sequence, homology of active site residues and three-dimensional structure.

Soybeans are known to cause allergies to some sensitive individuals. The allergenicity of genetically modified soybeans was assessed in relation to conventional varieties. Known allergenic proteins of soybeans were found to be unchanged, based on an evaluation of protein extracts from non-toasted, defatted soy flour. Assessing the allergenicity of proteins that are not derived from allergenic sources is more problematic, as discussed above. CP4 EPSPS fits one of the criteria common to allergenic proteins, that of molecular weight, but does not share any of the other characteristics. Table 4 shows the characteristics common to allergenic proteins.

Potential toxicity was assessed by considering the similarity of CP4 EPSPS to known protein toxins, an acute mouse gavage study and the study of the stability of CP4 EPSPS to digestion. First, CP4 EPSPS was not found to show any meaningful amino acid sequence homology when compared to known protein toxins in available databases. Next, an acute mouse gavage study was performed, which resulted in no adverse effects (body weight, cumulative body weight and food consumption) at a dose representative of a 1300-fold safety margin relative to the highest potential human consumption to the protein in a diet including genetically modified soybeans, corn, tomatoes and potatoes (assuming no loss in processing). An acute study was judged to be adequate in the toxicity assessment as proteins act as toxins by acute mechanisms. Finally, CP4 EPSPS was found to have a short half life in similated digestive fluids. The half life was measured as less than 15 seconds in gastric fluids and less than 10 minutes in intestinal fluids. The relatively short digestion time of the protein indicates a reduced likelihood that the protein would be toxic.

Finally, the prevalence of CP4 EPSPS in the diet was considered. As CP4 EPSPS was found to represent 0.025% of the extractable protein in soybean seed tissue, it was not expected to become a macroconstituent of the human or animal diet. The addition of the CP4 EPSPS gene was also not found to alter the carbohydrate, fat or oil composition, structure or levels of the soybean compared to conventional varieties, as described in the compositional analysis above.

Monsanto has published the research results that were submitted to FDA on the composition of glyphosate tolerant soybeans, toxicity, and feeding studies in a series of peer-reviewed articles in the Journal of Nutrition (Hammond, et al.; Harrison, et al.; Padgette, et al. 1996b). In addition, research results on the composition of glyphosate-tolerant soybeans treated with glyphosate, which were not originally submitted to FDA, were published in the Journal of Agriculture and Food Chemistry (Taylor, et al.).

Table 3. Animal Studies Submitted to FDA on Glyphosate Tolerant Soybeans

Animal	Feed	Duration of Study	Parameters Measured
Rats	Processed soybean meal	4 weeks	Mortality; Body weight; Cumulative Body weight gain; Organ weight; Food consumption
Rats	Unprocessed soybean meal	4 weeks	Mortality; Body weight; Cumulative body weight gain; Organ weight; Food consumption
Broiler chickens	Processed soybean meal	6 weeks	Body weight; Body weight gain; Feed intake; Feed/gain; Livability
Dairy cows	Raw soybeans	4 weeks	Milk production; Fat-corrected milk*; Milk composition; Dry matter; Net energy intakes; Body weight changes; Dry matter digestibility; Nitrogen balance; Volatile fatty acids in rumen; Rumen nitrogen
Catfish	Processed soybeans	10 weeks	Feed efficiency; Percentage weight gain; Survival; Food consumption*; Body composition; Moisture, protein, fat and ash in fillets
Bobwhite quail	Raw soybean meal	5 day	Mortality; Body weight gain; Food consumption

* statistical differences found between animals fed conventional soybean product and genetically modified soybean product.

Table 4. Characteristics of known allergenic proteins

Characteristic	Allergens	CP4 EPSPS
Molecular weight 10-70 kdal	yes	yes
Glycosylated	yes[a]	no
Stable to digestion	yes	no
Stable to processing	yes	no
Similar to known allergens	-[b]	no
Similar to soybean proteins	-	yes
Prevalent protein in food	yes	no

[a] Typically but not absolutely.
[b] Implicit for allergenic proteins from soybeans.
Source: Monsanto

B. Bt Corn

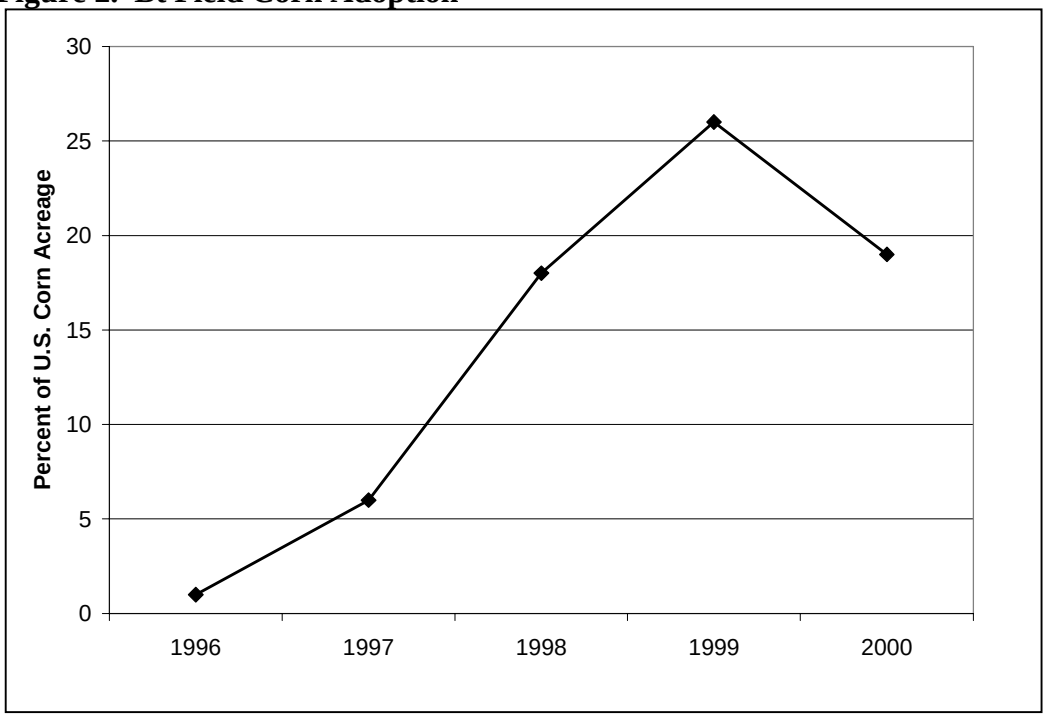
Genetically transformed plants incorporating genes from the soil bacterium *Bacillus thuringiensis* (Bt) were developed in the 1980's. Tobacco, cotton and tomatoes were among the first crop plants transformed to produce insecticidal protein to protect the plants from insect attack and the consequent yield losses. Bt corn varieties were developed and field tested by the early 1990's.

The first Bt field corn varieties were introduced for planting by U.S. farmers in 1996. Adoption of Bt corn varieties was swift, rising to 26% of corn acres planted by 1999, the fourth year on the market (USDA NASS 2000c). Adoption decreased to 19% in 2000, which is believed to be due largely to low target pest pressure in 1998 and 1999. Figure 2 shows adoption of Bt corn since its introduction in 1996.

Corn is the largest acreage crop grown in the U.S. Planted acreage totaled 80 million in 2000, which represents approximately one-quarter of the acreage planted to all crops in the U.S. Average corn yields totaled 134 bushels per acre in 1998 and 1999 (USDA NASS 2000a). Corn for grain production was estimated at 9 billion bushels in 1999 (USDA NASS 2000c) with a total value of production of $18 billion. This represents approximately 20% of the value of all crops grown in the U.S. (USDA NASS 2000d).

All 48 coterminous states have corn acreage, and, in many states, corn is the single most important crop in terms of acreage and production value. Corn production is concentrated in the Midwest, where ten states account for 85% of U.S. acreage and production. Individually, the states of Illinois and Iowa account for over 10 million acres of corn each (USDA NASS 2000a).

Figure 2. Bt Field Corn Adoption



Source: US EPA 2000a; USDA NASS 2000a

The major use of corn produced in the U.S. is as a livestock and poultry feed, accounting for 60% of use, while food, seed and industrial uses (including sweeteners, fuel alcohols and starch) account for approximately 19% of use. Exports account for the remaining 21% of use (USDA NASS 2000b).

The U.S. is by far the largest corn producing country, growing 40% of the world's total production. China is the second largest producer, growing 21% of the world's total. Brazil, Mexico, France and Argentina are also major corn producing countries. The U.S. dominates the corn export market, accounting for 75% of the world's total exports. Major export markets for U.S.-grown corn are Japan (29%), Korea (12%), Mexico (10%) and Taiwan (8%). Western Europe accounts for less than 1% of U.S. corn exports (USDA NASS 2000b).

Bt corn varieties were genetically modified to express an insecticidal protein from the soil bacterium *Bacillus thuringiensis* var. *kurstaki* (Cry proteins). The Cry protein used in Bt corn is selectively active against lepidopteran, or caterpillar insects, including the European Corn Borer (ECB), a major insect pest of field corn in the U.S. The ECB larvae ingest the Cry protein when it starts to feed on the plant. The toxin binds to the gut membranes, which causes the ECB larvae to die.

Several different types of Bt field corn have been approved by USDA, EPA and FDA. Table 5 shows the file numbers and dates of approvals by each agency for each

corn line that has been approved. These corn lines vary in the specific Cry protein that is produced, how much of the protein is produced, and where it is produced in the plant, which affects insect control. Most of the Bt field corn lines incorporate the CryIA(b) protein. StarLink contains the Cry9C protein and DeKALBt produces the CryIA(c) protein. Of all of the different corn lines that have already been approved for commercialization, only two will continue to be marketed: Syngenta Seeds' Bt11 and Monsanto's MON810. Registrations covering all other lines have been voluntarily cancelled or will not be renewed upon expiration. The most recent cancellation was for StarLink varieties, whose EPA registration was voluntarily withdrawn by Aventis in October 2000. StarLink varieties had been planted on less than 1% of total U.S. corn acreage. For the purposes of the current report, the focus is limited to the two lines that have continuing registrations: Bt11 and MON810.

Table 5. Bt Corn Approvals

Commercial Name	Description	Bt type	Company	APHIS Petition #	APHIS Approval Date	FDA File #	FDA Final Consultation Date	EPA Registration #	EPA Approval Date
KnockOut	Event 176	Cry1A(b)	Ciba-Geigy/ Novartis/ Syngenta	94-319-01	5/17/95	BNF24	1995	66736-1[1]	8/95
NatureGard	Event 176	Cry1A(b)	Mycogen/Dow					68467-1[2]	8/95
(Not commercialized)	MON801	Cry1A(b)	Monsanto	95-093-01	8/22/95	BNF18	1996	524-492[3]	5/96
YieldGard	Bt11	Cry1A(b)	Northrup King/ Novartis/ Syngenta	95-195-01	1/18/96	BNF17	1996	67979-1	8/96
YieldGard	MON810	Cry1A(b)	Monsanto	96-017-01[4]	3/15/96	BNF34[4]	1996	524-489	12/96
DEKALBt	DBT418	Cry1A(c)	DeKalb	96-291-01	3/28/97	BNF40	1997	69575-2[5]	3/97
(Not commercialized)	MON802	Cry1A(b)	Monsanto	96-317-01	5/27/97	BNF35[6]	1996		
Starlink	CBH351	Cry9C	AgrEvo/Aventis	97-265-01	5/8/98	BNF41	1998	264-669[7]	12/98

[1] Registration will expire on 4/1/01 and will not be extended.
[2] Registration will expire on 6/30/01 and will not be extended.
[3] EPA approval included lines MON801 and MON810. May 1996 approval for seed increase only. Registration voluntarily cancelled 5/98.
[4] APHIS approval and FDA consultation included lines MON 809 and MON810.
[5] Voluntary cancellation requested 4/18/00.
[6] FDA consultation included MON802, MON805, MON830, MON831 and MON832.
[7] EPA approval of Cry9C for feed use only. Registration voluntarily cancelled 10/00.

1. EPA

EPA is the lead agency in the regulation of Bt corn. EPA and APHIS jurisdictions overlap for consideration of environmental issues such as weediness, outcrossing, non-target impacts, environmental fate and insect resistance management.

EPA granted conditional registrations for both Bt11 and MON810 corn lines, as described further below, pending further non-target toxicity studies and development and implementation of insect resistance management plans. These registrations were originally scheduled to expire in April 2001. EPA announced in August 2000 that the time-limited registrations for Bt corn would be extended from April 2001 to September 2001 in order to assure that the renewal decisions were based on the most current health and ecological data (US EPA 2000b).

Over the past several years, EPA has convened numerous Science Advisory Panel (SAP) meetings to garner the advice of prominent scientists on the best scientific understanding of the impacts on health and the environment of regulatory actions concerning plant pesticides. A February 1998 SAP addressed issues related to insect resistance management (IRM). In a December 1999 meeting, the characterization and non-target organism data requirements were considered. The allergenicity of the Cry9C protein was the subject of meetings in February and November 2000. Mammalian toxicity assessment was addressed in a September 2000 SAP meeting.

The Agency is currently reviewing available data in their re-registration process. In October 2000, the Agency held another SAP meeting, broadly addressing the benefits and risks of the Bt crops, including corn, cotton and potatoes.

Bt11

On November 1, 1995, EPA announced in the Federal Register that Northrup King had submitted an application to register insect resistant corn produced using genetic material contained in plasmid vector pZO1502. Plasmid vector pZO1502 contains the CryIA(b) gene conferring insect resistance and a gene from *Streptomyces viridochromogenes* that codes for the production of phosphinothricin acetyltransferase (PAT), which confers resistance to the herbicide glufosinate. The application from Northrup King was approved on May 14, 1996 for seed propagation only. This registration was amended on August 5, 1996 to include commercial use in field corn. Registration was conditional upon data development in the area of resistance management and non-target toxicity. The registration, which was originally set to expire in May 1997, was amended to extend the expiration date to April 1, 2001 (US EPA 1997a).

On February 14, 1996, EPA announced the establishment of temporary exemptions from requirements of tolerance for CryIA(b) and for the pesticide inert ingredient phosphinothricin actyltransferase (PAT), and the genetic material necessary for their production (plasmid vector pZO1502) that were set to expire on May 30, 1996 (US

EPA 1996c). These temporary exemptions were later extended to April 17, 1997 (US EPA 1996d). On August 2, 1996, EPA announced the establishment of a permanent tolerance exemption for CryIA(b) and the genetic material necessary for its production in all plants, in response to a petition by Monsanto; this exemption applies to Bt11 corn also. The same day, EPA also announced a permanent exemption from the requirement of a tolerance for phosphinothricin acetyltransferase (PAT) and the genetic material necessary for its production in corn (plasmid vector pZO1502) (US EPA 1996e). This tolerance exemption was superseded on April 11, 1997, with the issuance of a permanent tolerance exemption for PAT and the genetic material necessary for its production in all plants (US EPA 1997c).

Toxicity

The toxicity of CryIA(b) is addressed below, as the relevant exemption from a requirement of a tolerance was granted pursuant to an application by Monsanto. Regarding the PAT protein, the Agency expects proteins with no significant amino acid homology to known mammalian protein toxins, which are readily inactiviated by heat or mild acidic conditions and readily degraded in an in vitro digestibility assay, to have little likelihood for displaying oral toxicity. The in vitro digestibility studies indicate that the PAT enzyme would be rapidly degraded following ingestion. Further, the PAT enzyme was shown to have no significant amino acid homology to known mammalian protein toxins. The PAT protein was also exhibited no toxicity in acute oral rodent studies.

Allergenicity

The allergenicity of CryIA(b) is addressed below, as the relevant exemption from a requirement of a tolerance was granted pursuant to an application by Monsanto. Allergenicity of the PAT protein was not considered to be a concern, as the protein has been found to be rapidly degraded in the gastric environment and lacks the chraacteristics common among allergenic proteins. (See Table 4 in Roundup Ready soybean section.)

Environmental fate

Data relevant to assessing the environmental fate of CryIA(b) protein included the results of laboratory studies, and field data. In a laboratory study, the bioactivity of CryIA(b), added to soil in corn tissue had an estimated half life of 1.6 days and an estimated DT90 of 15 days. The bioactivity of CryIA(b) in corn tissue incubated without soil had an estimated half life of 25.6 days, and a DT90 of 40.7 days. The bioactivity of purified CryIA(b) protein had an estimated half life of 8.3 days and a DT90 of 32.5 days.

Bt protein is expressed throughout the corn plant, but is concentrated in the leaves, where concentrations range from 10-168 ng CryIA(b) protein/mg total plant protein (uncorrected for extraction efficiency). Levels of CryIA(b) protein decrease as the plant reaches full maturity and begins to senescence.

Estimated total CryIA(b) protein per acre of corn is 0.57 pounds based on a total biomass of 89,300 lbs at physiological maturity. The highest concentrations are in the leaf at approximately 3.3 ug/g fresh weight. CryIA(b) protein degrades in soil over time (1-3 weeks), apparently at a faster rate than total plant protein. After one week, approximately 1% and 10% of the original levels of CryIA(b) protein remained in leaf and stalk tissue, respectively. After three weeks, CryIA(b) protein was still detected in the stalk tissue, but the level in transgenic leaves was similar to the background levels seen in control leaf tissue. CryIA(b) protein apparently binds to soil particles, making quantitative extraction difficult. Biological activity, assessed by European corn borer bioassay, is reduced to control levels after three weeks of incubation in soil.

Weediness

Increased weediness of Bt11 corn compared to conventional corn varieties was judged to be unlikely. This conclusion was based on the assessment that cultivated corn is not considered a weed pest and that there was no indication that the incorporation of the CryIA(b) gene or the herbicide tolerance gene would convert cultivated corn into a weed. Corn cannot maintain itself outside of cultivation as a weed. No weediness differences were observed in trials. Data provided in the Northrup King petition on seed germination rates, yield characteristics, disease and pest susceptibilities, and compositional analyses were judged to be sufficient for a determination that Bt11 corn would not be more weedy than conventional corn varieties.

Out-crossing

Closely related annual teosinte and corn are wind pollinated, tend to outcross, and are highly variable, interfertile species. Corn and teosinte are genetically compatible, and, in areas of Mexico and Guatemala, they freely hybridize when in proximity to each other. A frequency of one F1 hybrid for every 500 corn plants or 2 to 5% of the teosinte population for the Chalco region of the Valley of Mexico has been reported. The F1 hybrid of teosinte by corn is robust and fertile and is capable of backcrossing to corn. Teosinte is not present in the U.S. Corn Belt, as its natural distribution is limited to the western escarpment of Mexico and Guatemala and the Central Plateau of Mexico.

Tripsacum species, may also be crossed with corn, though the generation of tripsacum-corn hybrids requires special techniques. Except for *Tripsacum floridanum*, it is difficult to cross *Tripsacum* with corn, and the offspring display varying levels of sterility. *Tripsacum floridanum* is native to the southern tip of Florida. Other *Tripsacum* species are native to Mexico, Guatemala and South America.

All corns will interpollinate, except for certain popcorn varieties and certain hybrids. Pollen of a specific hybrid can be carried by wind to pollinate other dent corn hybrids, sweet corn, and popcorn, if the popcorn does not carry the dent-sterile trait.

Outcrossing of transformed corn plants with wild relatives of corn will be the same as for non-transformed corn plants. Outcrossing with teosinte species will only occur where teosinte is present in Mexico and Guatemala. Outcrossing with Tripsacum species is not known to occur in the wild and only under very careful conditions can corn be crossed with Tripsacum. The possibility exists, though unlikely, that the exchange of genes would occur between corn and its wild relatives. No cases of gene flow between corn and its wild relatives are known in the U.S.

Gene exchange between cultivated corn and transformed corn would be similar to what naturally occurs at the present time. The chances that a weedy type of corn will result from outcrossing with cultivated corn was judged to be extremely remote.

Expression of the insect control protein will not likely provide a competitive advantage sufficient to cause these to be any more "weedy" than other corn cultivars. Northrup King submitted field data that APHIS judged as demonstrating that Bt11 corn is no more weedy than the non-modified recipient. The phosphinothricin resistance trait used as a selectable marker in Bt11 corn was considered not to increase the potential weediness of Bt corn, because the trait confers no advantage to the plants unless they are sprayed with the herbicide. At the time of original registration, Bt11 corn was not approved for use with the herbicide glufosinate.

Non-target impacts

The assessment of non-target impacts relied upon studies conducted on several insect and other species. The results of submitted toxicology studies are summarized in Table 6. The original petition by Northrup King was judged to be lacking in data to address potential impacts on Collembola, a soil organism, and *Daphnia magna*, an aquatic invertebrate. Additional data were requested by the Agency as part of the conditions on the registration. Although the Agency requested further data to address toxicity to Collembola and *Daphnia magna*, there are no threatened or endangered soil invertebrates that are closely related to Collembola, and no threatened or endangered arthropods that are closely related to *Daphnia magna*. The overall risk to populations of these organisms was anticipated to be minimal during the period of the conditional registration. Studies demonstrating lack of adverse effects on *D. magna* and Collembola have since been provided by the registrant.

Since the approval of Bt corn, concern has been raised about the impacts of Bt corn pollen on monarch butterfly populations, in response to results of a laboratory study showing toxicity of Bt corn pollen to monarch butterfly larvae feeding on pollen coated milkweed. EPA concluded that the results of this and one other controlled study were not useful in the risk assessment of widespread or recurring Bt corn pollen effects on monarch butterflies without additional field study. Additional field studies were requested to address: the distribution of butterflies, milkweed plants and corn; corn pollen release and distribution in the environment; toxicity of Bt corn Cry proteins and Bt corn pollen to lepidopterans; monarch egg laying and feeding behavior; and monarch

population monitoring (US EPA 2000a). EPA did not specifically address the potential impact of Bt corn on the monarch butterfly in its initial approval process. The Agency did consider the possible impact upon threatened or endangered lepidopteran insects and other species, concluding that there would be no exposure by these organisms to the CryIA(b) protein.

A considerable amount of research has been conducted since the results of the monarch laboratory study were published, the results of which are still being collected and interpreted. In December of 1999, EPA issued a data call in for non-target lepidopteran effects, with data due in March 2001. The registrants have already submitted information in response to the data call in. This information has been reviewed by EPA and a preliminary assessment of the available data has been issued. The Agency concluded that "…the published preliminary monarch toxicity information is not sufficient to cause undue concern of widespread risks to monarch butterflies at this time" (US EPA 2000a). More data will be submitted to the agency prior to the March 2001 deadline. This information, while not required as a condition of registration, is being considered by EPA in its assessment for renewed registration of Bt corn.

Resistance Management

Northrup King proposed a strategy to maximize the utility of Bt11 corn while delaying the development of insect resistance to these plants, including: incorporation of Bt11 into IPM programs; monitoring; high dose expression; refugia; development of new insect control proteins with a distinct mode of action to be employed with the CryIA(b) protein; and the implementation of a grower education program.

IRM is another area where research is continuing, in order to develop appropriate strategies. EPA required further research on IRM as a condition of registration, as described below. IRM was the subject of a Science Advisory Panel meeting in 1998. EPA and USDA held a joint workshop on resistance management in June of 1999, convening over 150 representatives from academia, industry, grower and public interest groups, federal and state government agencies, and the general public. The workshop included four panels to address various aspects of IRM implementation for Bt corn (Stewart).

In April 1999, Bt corn registrants announced the voluntary implementation of a uniform, industry-wide Bt corn IRM plan for 2000, a year ahead of the schedule EPA required (see below under conditions of registration). This plan made the planting of a non-Bt refuge mandatory of each corn grower (National Corn Growers Association).

New measures for IRM were announced by the Agency in December 1999, for the 2000 season, requiring minimum refuges, monitoring, and voluntary measures for the protection of non-target insects, such as the Monarch butterfly. These new measures largely made mandatory refuge requirements that had previously been voluntary and left to the registrants to enforce.

Conditions of the registration

The Northrup King petition for Bt11 was granted on conditions that included: expiration on April 1, 2001; use for field corn only; development of a draft plan for "structured" refugia by 8/9/98 and a final plan by 1/31/99; implementation of the structured refuge plan by April 1, 2001; monitoring for development of resistance; reporting of resistance; grower education; establishment of a resistance management research program; limits on amount of seed sold in cotton growing areas; and the submission of collembola and Daphnia magna studies by 5/14/97. Syngenta Seeds (formerly Novartis Seeds) has submitted a Daphnia magna study and cited a collembola study conducted by Monsanto, with the permission of EPA. EPA requested in the Fall 2000 that Novartis (now Syngenta) submit an additional collembola study, using a different test material.

Table. Results of Non-target Toxicity Studies for Bt Field Corn

Non-target organism	Test results
Honey bee larvae	Practically nontoxic; NOEL > 20 ppm
Honey bee adults	Mean mortality 16.2%, not significantly different from control; NOEL < 20 ppm
Parasitic hymenoptera	Practically nontoxic; NOEL > 20 ppm
Green lacewing larvae	Practically nontoxic; NOEL > 16.7 ppm
Lady beetles	Practically nontoxic; NOEL > 20 ppm
Northern bobwhite quail	No treatment related mortality or difference in food consumption, body weight or behavior at 50,000 ppm or 100,000 ppm corn meal from Bt corn
Earthworm	NOEL > 200 ppm
Collembola	No observable toxicological effect at 200 ppm[1]
Collembola[2]	LD50 of lyophilized MON810 corn leaf tissue over 28-day exposure period is greater than 50% by weight of diet.
Channel catfish	No significant differences in feed/fish, feed conversion ratios, final weight, % weight gain, survival. No differences in body composition (% moisture, fat and ash). Higher protein content of fish on dry weight basis.
Daphnia magna[2]	Unaffected by 48 hour exposure to 100 mg of Bt corn pollen/liter.

[1] Original study submitted in support of Bt11 petition. Study conducted using bacterially produced CryIA(b); EPA requested additional data using lyophilized leaf extract as a condition of registration.
[2] Study submitted to satisfy EPA request for additional data.
Sources: US EPA 1996h; US EPA 2000a

MON810

EPA published a notice on October 25, 1995, announcing the submission by Monsanto of a petition requesting an exemption from the requirement of a tolerance for Bt CryIA(b) protein as produced in plant cells. On July 31, 1996, EPA announced the establishment of temporary exemptions for CryIA(b) and CP4 EPSPS and the genetic material necessary for their production that were scheduled to expire on April 25, 1997

(US EPA 1996g). There were no adverse comments in response to this notice. The petition was approved effective August 2, 1996 (US EPA 1996f).

On April 17, 1996, EPA issued a Federal Register notice announcing the submission of an application by Monsanto to register the pesticide product CryIA(b). A conditional registration for seed propagation only was granted on May 29, 1996 for corn line MON801. On July 16, 1996, this registration was amended to allow plantings of corn line MON810 (US EPA 1996h). Full commercial use was approved on December 20, 1996, conditional upon development of data on resistance management and Collembola and Daphnia magna toxicity. The registration was originally set to expire on April 1, 2001 (US EPA 1997b).

MON810 was transformed using vectors containing genes encoding CryIA(b), CP4 EPSPS, GOX and nptII. However, molecular analysis of line MON810 established that the line does not contain the CP4 EPSPS, gox or nptII genes. Much of the details on the safety of MON810 were summarized and published by Monsanto (Sanders, et al.).

Toxicity

The toxicity of CryIA(b) was assessed in consideration of granting an exemption from the requirement of a tolerance under FFDCA. Submitted information included characterization of the expressed protein, the acute oral toxicity and in vitro digestibility of the protein. No test substance related deaths occurred at a relatively high dose in an acute mouse oral toxicity test using bacterially derived CryIA(b) protein. The dose used in the study represented a dietary exposure margin of 22 million (Betz, et al.). Information was submitted to show that the test material derived from microbial cultures was biochemically and insecticidally similar to the delta-endotoxin as produced by the plant-pesticide in corn. Proteins with no significant amino acid homology to known mammalian protein toxins and which are readily inactivated by heat or mild acidic conditions and are readily degraded in an in vitro digestibility assay would have little likelihood for displaying oral toxicity. Toxic proteins are known to act via acute mechanisms and at very low dose levels. The agency considered CryIA(b) protein to not be toxic since no acute effects were observed, even at relatively high dose levels. The in vitro digestibility studies indicated that the delta-endotoxin would be rapidly degraded following ingestion. The genetic material necessary for the production of the *Bacillus thuringiensis* CryIA(b) delta-endotoxin were judged to not pose any potential toxicity, as DNA is common to all forms of plant and animal life and the Agency knows of no instance where these nucleic acids have been associated with toxic effects related to their consumption.

Allergenicity

The allergenicity of CryIA(b) was also assessed in consideration of granting an exemption from the requirement of a tolerance under FFDCA. Current scientific knowledge suggests that common food allergens tend to be resistant to degradation by

heat, acid, and proteases, are glycosylated and present at high concentrations in the food. Data were submitted demonstrating that the CryIA(b) protein is rapidly degraded by gastric fluid in vitro and is non-glycosylated. In addition, studies on laboratory animals have not indicated any potential for allergic reactions to Bt or its components, including the delta-endotoxin in the crystal protein. Recent in vitro studies also confirm that the delta-endotoxin would be readily digestible in vivo, unlike known food allergens that tend to be resistant to degradation. Bt pesticides have been registered since 1961 and have been widely used. In that time, there have been no confirmed reports of immediate or delayed allergic reactions to the delta-endotoxin, despite significant oral, dermal and inhalation exposure to the microbial product.

Gene flow

Information on weediness and gene flow of MON810 was similar to that provided for Bt11 corn.

Environmental Fate

In addressing the environmental fate of the CryIA(b) protein, the same laboratory studies are relied upon as discussed above for Bt11 corn.

1994 field data demonstrated expression levels of 0.18-0.39 ug/g in grain, 7.93-10.34 ug/g in the leaf, 3.65-4.65 ug/g in the whole plant, and 0.09 ug/g in the pollen. Marker gene products were undetectable. 1995 field data were consistent with 1994 field data, showing that expression was stable from 1994 to 1995.

Non-target

Monsanto submitted the same non-target studies that were cited above by Northrup King in the Bt11 petition.

Resistance Management

Monsanto cited Northrup King's resistance management plan to support their registration application. Monsanto's Bt corn is similar to Northrup King's previously registered Bt corn product because they both express the CryIA(b) delta-endotoxin in kernels and silks.

Conditions of the registration

EPA granted a conditional registration for MON810 similar to that for Bt11. Monsanto submitted the required non-target toxicity studies on collembola and Daphnia, which were judged to be satisfactory to addressing the Agency's concerns.

2. USDA

Due to the overlapping jurisdiction between EPA and USDA on environmental issues, much of the same information was considered by the two agencies in their approvals. Therefore, while USDA considers environmental impacts such as weediness, outcrossing, non-target effects and insect resistance management, since those issues were discussed previously, they will not be addressed here. Additional information specific to USDA's approval is provided.

Bt11

APHIS received a petition requesting a determination of nonregulated status for Bt11 insect-protected corn from Northrup King on July 14, 1995. APHIS announced receipt of Northrup King's petition in the Federal Register on September 7, 1995, stating that the petition was available for public review and comment (USDA APHIS 1995b).

APHIS received 106 comments on the Northrup King petition from seed companies, individuals, farmers and farm seed dealers, agricultural products companies, State departments of agriculture, an agricultural council, a growers association, and a university. All comments were favorable to the petition.

The genetic material incorporated into corn line Bt11 includes a gene encoding the active ingredient protein from the soil microorganism, *Bacillus thuringiensis,* and a gene encoding resistance to the herbicide glufosinate, for assistance in selecting successfully transformed plants, derived from the soil microorganism *Streptomyces viridochromogenes*.

Bt11 is tolerant to the herbicide glufosinate, but is not registered for use with glufosinate. Herbicides were used to select successfully transformed plants. The ampicillin resistance gene was used as a selection marker for bacteria containing the genetic construct, but the genetic sequence is not present in the plant.

Bt11 corn was considered a "regulated article" because it contains components from organisms that are known plant pathogens, i.e. the cauliflower mosaic virus and the bacterium *Agrobacterium tumefaciens*. Bt11 field corn had been field tested since 1992 in the major U.S. corn growing regions with APHIS approval. Three permits were issued and 42 notifications were acknowledged to allow field testing under physical and reproductive confinement.

The Northrup King petition provides information describing the genetically modified corn plants and information relevant to determining whether Bt11 corn plants are more likely than conventional varieties to become a plant pest. The petition includes a contribution from a University of Illinois entomologist that describes the anticipated benefits associated with planting corn genetically modified to resist the European corn borer and other lepidopteran pests. Next a description of corn biology is provided.

Experimental data is presented on: the yield effects of the genetic transformation; determination that the ampicillin resistance gene is not present; efficacy towards European corn borer and other lepidopteran pests; inheritance and gene stability; insertion site; insert copy number; expression of the encoded protein; quantity of Bt protein produced per acre; environmental fate; and the presence of PAT. The petition then describes the genotype of Bt11 corn in comparison to conventional corn. The differences in phenotype are addressed with a presentation of data from field trials, comparing disease and insect susceptibility, weediness, stand, phenotype, soil organisms, beneficial insect populations. No differences in these characteristics were observed in 62 trials, with two exceptions where slight differences in susceptibility to the plant disease Stewart's wilt were observed.

The petition addresses potential environmental consequences of unregulated release of Bt11 field corn, including enhanced weediness of the transformed corn plant, gene flow, impact on processed commodities, impacts on non-target and beneficial organisms and endangered species, and plant pathogenicity. Finally, as appendices to the petition, USDA field reports for trials conducted in 1992-1994 are included, as well as a draft label statement regarding glufosinate resistance, a recommended approach to insect resistance management, details on the methods used to measure Bt expression in the plant, and results from a study addressing the equivalence of the plant and microbially produced Bt proteins (Pilacinski, et al.).

APHIS reached conclusions similar to EPA regarding weediness, outcrossing, and non-target impacts. With respect to insect resistance management, APHIS notes that the registrant has developed a strategy to address the issue, and defers to regulation in this area by EPA. In the determination of non-regulated status, APHIS concluded that Bt11 corn exhibits no plant pathogenic properties. Although DNA from pathogenic organisms were used in their development, these corn plants are not infected by these organisms nor can these plants incite disease in other plants. Further, Bt11 corn was not expected to have any adverse impact on raw or processed plant commodities. Bt11 corn exhibited typical agronomic characteristics of the recipient plant, with the exception of the desired phenotype conferred by the Bt insect control protein. In APHIS' opinion, the components, quality and processing charcteristics of Bt11 corn reveal no differences that could have an indirect plant pest effect on any raw or processed plant commodity.

MON810

Monsanto's request for approval of the two Bt corn lines, MON809 and MON810, was made in connection with a previous approval of insect resistant corn line MON80100, which was announced on September 5, 1995 (USDA APHIS 1995a). MON80100 was approved by APHIS, but was never commercialized. The same plasmid vectors were used in the generation of MON80100, MON809 and MON810. Information on these two lines was included in the original petition for MON80100. Additional information on these two corn lines was submitted to the Agency in a separate petition for

these two lines. As only MON810 was eventually commercialized, the current discussion is limited to this one line, ignoring any information submitted specifically on MON809.

APHIS received a petition requesting a determination of non-regulated status for insect-resistant corn line MON80100 on April 3, 1995. On June 7, 1995, APHIS published a notice in the Federal Register announcing that the Monsanto petition had been received and was available for public review. APHIS received nine comments on the Monsanto petition. All comments were favorable to the petition.

Monsanto's original petition for MON80100 begins with a description of the potential benefits of insect protected corn varieties by a University of Illinois entomologist. The petition continues with a description of corn biology, which addresses outcrossing and weediness issues, by an Iowa State University scientist. Next is a description of the transfer system and the plasmids that were utilized, the donor genes and the molecular analysis of MON80100. Finally, information describing the phenotype of MON80100 is provided. Appendices include a more detailed discussion of the benefits of Bt corn, USDA reports from field trials, an example of the field monitoring form and a discussion of Monsanto's recommended strategy for insect resistance management.

APHIS received a petition in support of non-regulated status for corn lines MON809 and MON810 on January 17, 1996 (USDA APHIS 1996). Monsanto's petition requesting non-regulated status for additional corn lines MON 809 and MON810 provides information specific to these two modified corn lines. A description of the potential benefits of insect protected corn is provided, followed by a brief section on the biology of corn, referring the earlier petition for MON80100. Detailed descriptions of MON809 and MON810 are given, on the transformation system and genetic material utilized, molecular analysis, and phenotype. Detailed information is given on: the analysis of expression of CryIA(b), CP4 EPSPS, GOX and NPTII; results of field germination tests; disease and pest susceptibility differences; yield characteristics and compositional analysis.

APHIS reached conclusions similar to EPA regarding weediness, outcrossing, and non-target impacts. With respect to insect resistance management, APHIS notes that the registrant has developed a strategy to address the issue, and defers to regulation in this area by EPA. In the determination of non-regulated status, APHIS concluded that MON810 corn exhibits no plant pathogenic properties. Although DNA from pathogenic organisms were used in their development, these corn plants are not infected by these organisms nor can these plants incite disease in other plants. Further, MON810 corn was not expected to have any adverse impact on raw or processed plant commodities. MON810 corn exhibited typical agronomic characteristics of the recipient plant, with the exception of the desired phenotype conferred by the Bt insect control protein. In APHIS' opinion, the components, quality and processing characteristics of MON810 corn reveal no differences that could have an indirect plant pest effect on any raw or processed plant commodity.

3. FDA

While the safety of CryIA(b) and the genetic material necessary for its production in plants is evaluated by EPA, all other issues that pertain to the food and feed safety of Bt corn fall within the compass of the FDA's review.

Bt11

Northrup King submitted information to FDA dated October 25, 1995 (Williams). The consultation summary includes descriptions of the host organism, the modified corn line, including a compositional analysis, the donor organism and the inserted genetic material and products.

In the compositional analysis, levels of protein, oil, starch and fiber were compared. Very small differences were observed and all values were within ranges established for corn in the literature. It was concluded that the levels of the components analyzed did not significantly change. A compositional analysis of silage was also performed. Measurements of dry matter, crude protein, available crude protein, acid dietary fiber, neutral dietary fiber, total digestible nutrients, calcium, phosphorus, potassium, and magnesium were provided. Statistical differences were found for acid dietary fiber, nutrient dietary fiber and total digestible nutrients in one test. In the other test, statistically significant differences were found for calcium and potassium. However, the values were within ranges normally seen for hybrid field corn.

MON810

Monsanto submitted information to FDA dated June 6, 1996. Information submitted included a description of the rationale for the development of insect-protected corn; a section on the benefits of corn modified to be resistant to ECB by a University of Illinois entomologist; molecular characterization of the modified corn lines; and a safety assessment based on compositional analysis, safety of donor organisms, and safety of introduced proteins.

The compositional analysis was based on comparisons of various components of the modified corn to conventional corn: proximate analysis, amino acid composition, fatty acid composition, inorganic components and forage composition. No differences were found between MON810 and the control corn line in any of the comparisons. The proximate analysis compared levels of the major components of corn grain: protein, fat, ash, carbohydrates, calories and moisture. The levels of these components were equivalent for line MON 810 and the control line. Amino acid composition was also assessed in comparison to a control. The values as percent of total protein for 18 amino acids were comparable for MON 810 and the control. The values for fatty acid composition were measured for detectable fatty acids. These values were also similar for MON 810 and the control. The levels of the inorganic components calcium and phosphorus were measured and were comparable for MON810 and the control. The

components of the forage (protein, fat, ash, carbohydrates, calories and moisture, acid detergent fiber and neutral detergent fiber) were compared and found to be similar for MON810 and the control line (Croon, et al.).

III. Benefits

A. Roundup Ready Soybeans

The primary reason growers have adopted Roundup Ready weed control programs is the simplicity of a weed control program that relies on one herbicide to control a broad spectrum of weeds without crop injury or crop rotation restrictions. Before the introduction of Roundup Ready soybean varieties, growers would choose between many herbicides, often applying three or more active ingredients, some of which would cause damage to the growing soybean plants, or cause harm to corn crops that commonly follow soybeans (Gianessi and Carpenter).

Roundup is a highly effective broad spectrum herbicide that controls both broadleaf and grass weeds. Each year, state extension services release weed control guides for field crops including soybeans. The guides provide information on the efficacy of available herbicide treatments on specific weed species, as well as ratings of crop safety. In the Michigan State University weed control guide, in which 182 treatments are rated on 24 different weed species, Roundup, used over Roundup Ready soybeans received the 23 good or excellent ratings. In addition, the Roundup treatment is rated with a minimal risk of crop injury. The next best available treatment with similar crop safety received 16 good or excellent ratings (Kells, et al.).

Growers also have more flexibility in timing herbicide treatments with the Roundup Ready system. Maximum weed heights at which Roundup is effective on most weed species are higher than other available herbicides. This allows growers to treat later if needed and still get effective weed control. Further, some commonly used soybean herbicides may cause injury to rotation crops. Because of this potential for injury to crops following soybeans, rotation restrictions are specified on the labels of these herbicides. For instance, sugarbeets may not be planted for 40 months after a field is treated with imazethapyr, a commonly used soybean herbicide.

The primary impacts of adopting Roundup Ready weed control programs include changes in costs and pesticide use. Roundup Ready programs were introduced to be price competitive with existing conventional programs. The introduction of competitively-priced Roundup Ready programs resulted in manufacturers of other products dropping their prices, in some cases by 40%. This resulted in an estimated $216 million cost savings for soybean growers in 1999 compared to 1995, the year before Roundup Ready varieties were introduced, including the technology fee paid by growers who planted Roundup Ready varieties. Table 7 shows estimated soybean weed control program costs for 1995, 1998 and 1999.

Table 7. Soybean Weed Control Costs

	1995	1998	1999
		(millions)	
Herbicide Expenditures	$1,865	$1,482	$1,441
Technology Fee	$0	$160	$208
Net Weed Control Costs	$1,865	$1,642	$1,649

Note: Calculated assuming herbicide expenditures in 13 states represent 80% of U.S. total.

Herbicide use in soybeans has been affected dramatically by the introduction of Roundup Ready soybean varieties. The US Department of Agriculture estimates the total number of acres treated and number of treatments by herbicide each year. The total number of pounds of herbicides used per soybean acre was the same in 1999 as in 1995, the year before Roundup Ready soybeans were introduced (Table 8). The mix of herbicides being used in soybeans has changed. As one would expect, the use of glyphosate has increased, from being used on 20% of acreage in 1995 as a burndown or spot treatment, to being used on 62% of acres in 1999. The use of other herbicides has decreased. Imazethapyr, the most widely used soybean herbicide in 1995, was used on 44% of soybean acres in 1995, compared to 16% in 1999. Figure 3 shows trends in herbicide use and acreage for 1990 through 1999 for eight states. Growers have also reduced the number of herbicide applications. Comparing 1995, the year before Roundup Ready varieties were introduced, and 1999, the last year for which data are available, the number of herbicide applications has decreased by 19 million, or 12%.[1] These changes in herbicide use occurred even though the total number of soybean acres increased by 18% between 1995 and 1999. The decrease in herbicide applications demonstrates growers using fewer active ingredients and making fewer trips over the field, which translates into ease of management.

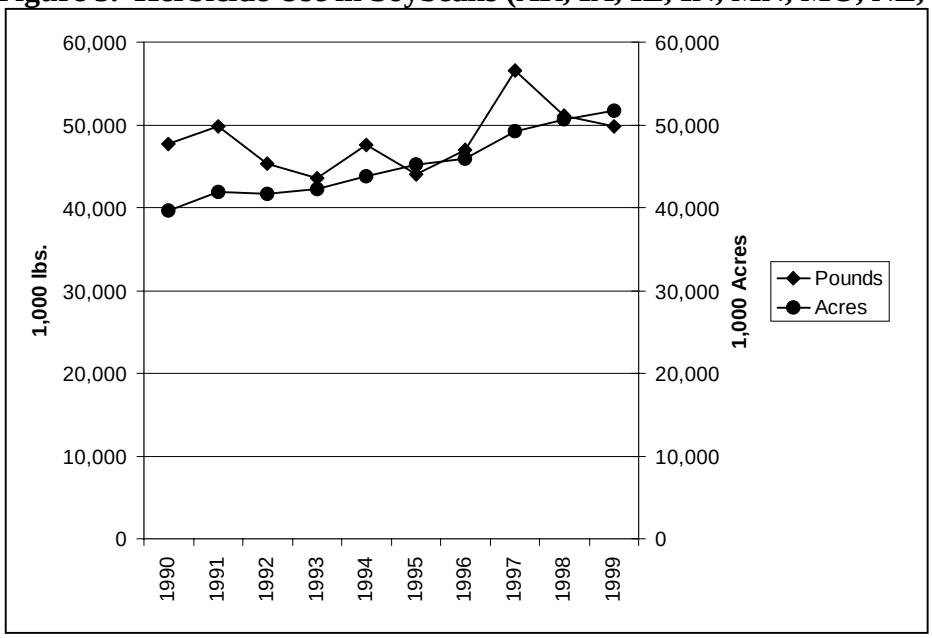
[1]An application is the number of different active ingredients applied per acre times the number of repeat applications, and differs from the number of trips over the field, as one trip across the field to apply two active ingredients is treated as two applications, as is two treatments each containing a single ingredient.

Table 8. Soybean Herbicide Application Rates (AR, IA, IL, IN, MN, MO, NE, OH)

Year	Rate (lbs/acre)
1990	1.26
1991	1.23
1992	1.11
1993	1.06
1994	1.11
1995	1.00
1996	1.05
1997	1.18
1998	1.06
1999	1.00

Source: USDA NASS 1991-2000

Figure 3. Herbicide Use in Soybeans (AR, IA, IL, IN, MN, MO, NE, OH)



Source: USDA NASS 1991-2000

B. Bt Corn

Bt corn varieties have allowed growers to control a pest that had been largely untreated previously. The use of Bt corn has resulted in higher yields and a modest reduction in the amount of insecticides used.

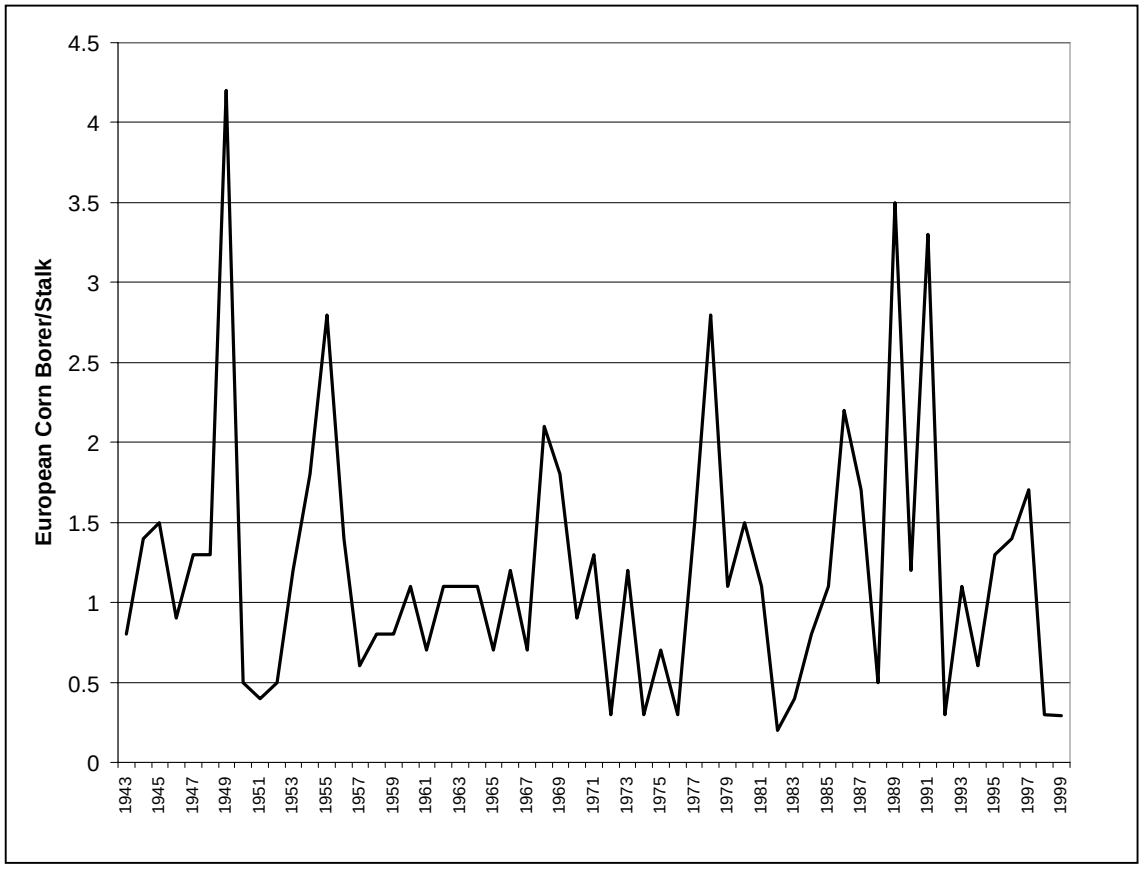
Due to the difficulty in scouting for the European corn borer (ECB) and the importance of timing insecticide application before the caterpillar bores into the corn stalk and is protected from insecticides, it is estimated that less than 5 percent of U.S. field corn acreage was being treated with insecticides for the ECB prior to the introduction of Bt corn varieties.

Researchers have sought alternative methods to control the corn borer, although none has proven effective on a wide scale. Traditional breeding efforts to select for natural resistance to the corn borer resulted in the development of varieties with intermediate levels of resistance that were widely used into the mid 1970s. However, acreage planted to these varieties decreased dramatically due to the introduction of much higher-yielding susceptible hybrids, which could produce higher yields than the resistant hybrids, even after sustaining high levels of damage from the corn borer. Another extensive research program was undertaken by the U.S. Department of Agriculture (USDA) to identify natural predators of the corn borer. The program resulted in the introduction of 24 species of parasites in U.S. corn production areas, of which only six became established. While these beneficial insects have provided control of the corn borer in some years in some areas, their impact has been limited.

The impacts of Bt corn include increased yields and reduced pesticide use. However, the main advantage of Bt field corn has been increased yields, as only a small proportion of U.S. field corn acreage was sprayed for the ECB prior to the introduction of Bt varieties, as noted above.

Yield losses due to the corn borer vary from year to year with infestation levels, which are generally unpredictable from one year to the next. Figure 2 shows ECB infestation levels in Illinois for 1943 to 1999. Largely uncontrolled until the introduction of Bt corn varieties, the ECB has caused production losses that have ranged from 33 million bushels to over 300 million bushels per year (USDA APHIS 1975). Bt corn varieties have been shown to provide a very high level of protection from the corn borer. Plants sustain only minute damage as the corn borer larvae attempt to feed.

Figure 2. European Corn Borer Densities in Illinois 1943-1999



Sources: Briggs, et al.; Gray, et al.; Monsanto; Steffey

The benefits that growers realize from planting Bt corn varieties depend on the level of infestation. In light infestation years the benefits may not be great, while in heavy infestation years growers will realize substantial yield increases. An average of available research results comparing yields from Bt and non-Bt corn fields indicates that growers experienced a yield advantage of approximately 12 bushels an acre in 1997, 4.2 bushels an acre in 1998 and 3.3 bushels an acre in 1999. The seed price premium, or technology fee, for Bt corn was approximately $10 an acre in 1997 and 1998 and $8 in 1999. Assuming corn prices of $2.43 a bushel in 1997, $1.95 a bushel in 1998 and $1.90 a bushel in 1999, the average income changes for Bt corn were an increase of $18 an acre in 1997, a decrease of $1.81 an acre in 1998 and a decrease of $1.73 an acre in 1999. It is expected that in 10 of the 13 years from 1986 to 1998, corn borer infestations in the Corn Belt were such that corn growers would have realized a gain from planting Bt corn (Monsanto 1999). Table 9 shows aggregate cost and benefits estimates of Bt corn for 1997 through 1999.

The greatest impact of Bt corn varieties is the increase in production it provides through reduced yield losses due to the ECB. Table 10 shows the increase in production

due to reduced yield losses due to Bt corn for 1997 through 1999. In 1999, growers were able to reduce their yield losses by 66 million bushels, the equivalent to production on nearly 500,000 acres of corn production that would otherwise have been destroyed by the corn borer.

Reductions in insecticide use are also expected due to the introduction of Bt corn varieties, though these reductions are anticipated to be modest due to low levels of insecticide use for ECB prior to the introduction of Bt corn. Attributing any observed changes in insecticide since 1995 to the introduction of Bt corn is necessarily problematic for several reasons. First, insecticides are typically used for control of several target pests. USDA pesticide use surveys do not report insecticide use by target pest, which means that isolating insecticide use targeted solely to the ECB is impossible. Second, insect populations by their nature are highly variable from year to year, which makes trends difficult to discern. Finally, the introduction of new products and development of resistance in insect populations to older products also drive shifts in insecticide use.

Five insecticides are currently recommended for control of ECB: Bt (foliar spray), chlorpyrifos, permethrin, lambdacyhalothrin, and methyl parathion (University of Illinois). With the exception of foliarly applied Bt products, these insecticides are typically used for several target pests, including cutworms, rootworms, armyworms as well as the ECB. Comparing 1995, the year before Bt corn varieties were introduced, to 1999, the use of these five insecticides declined. (See Table 11.) Of interest is the decline in use of four of the insecticides between 1995 and 1999: chlorpyrifos (-2%), permethrin (-1%), Bt (-1%), and methyl parathion (-2%). The decrease in the use of these insecticides may be due to the introduction of Bt corn varieties and the resulting reduced need for sprays targeted at the ECB. However, several other explanations are also possible to explain the change from 1995 to 1999. Lambdacyhalothrin was introduced in 1996, primarily for the treatment of cutworm in-season, which has likely displaced some of the use of the other insecticides, such as chlorpyrifos. Adult corn rootworm beetle populations have reportedly developed resistance to methyl parathion in some areas, which may account for part of the decline in its use. Finally, these reductions may be due to a decline in insecticide treatments targeted at pests other than the ECB. It is likely that all of these factors have affected use patterns of these insecticides.

The aggregate reduction of the percent acreage treated with the five insecticides is 6%. Assuming that 3% of the change is attributed to the introduction of lambdacyhalothrin, implies that a 3% decline occurred as a result of changes in the target pest complex, including ECB and other pests. For analytical purposes, it is assumed that one-half of the decline in the usage of the four insecticides (1.5%) was due to the introduction of Bt corn, implying over 1 million fewer acres sprayed for ECB.

Table 9. Aggregate Costs and Benefits of Bt Corn 1997-1999

	Costs	Benefits	Net Gain (Loss)
	(millions)		
1997	$47	$136	$88
1998	$144	$118	($26)
1999	$161	$126	($34)

Table 10. Aggregate Production Increases from Bt Corn 1997-1999

	Bushels/Acre	Total Bushels (1,000)
1997	11.7	55,832
1998	4.2	60,606
1999	3.3	66,436

Table 11. Corn Insecticides Used for European Corn Borer Control (Percent Acres Treated)

Active Ingredient	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999
	percent of acres treated									
Chlorpyrifos	6.1	9	8	8	8	7	8	7	6	5
Lambdacyhalothrin							2	1	2	3
Methyl Parathion		2	1	2	2	3	2	4	1	1
Permethrin	1.4	2	2	2	3	4	4	5	1	3
Bt (foliar spray)					1	1	1	1		

Source: USDA NASS 1991-2000

IV. Conclusions

Critics of agricultural biotechnology express objections to the commercialization of genetically modified crops, based on the belief that little is known concerning the potential impacts on health and the environment. However, it is clear from reviewing the information that was submitted in support of commercialization of these new varieties, that extensive studies were conducted addressing these risks. The three regulatory agencies reviewed the studies, and where information was deemed to be lacking but not critical, granted approval conditional upon submission of further information.

In reviewing the studies that were conducted on the safety of Roundup Ready soybeans, no indication of greater health or environmental risks were found compared to conventional varieties. The benefits of the introduction of Roundup Ready soybeans include savings of $216 million in annual weed control costs and 19 million fewer soybean herbicide applications per year.

The review of Bt corn studies submitted to support approval shows that no indication of greater health or environmental risks were found, although conditional registrations were granted at the time of commercialization pending the submission of additional studies on non-target impacts and further development of insect resistance management programs. These registrations are currently under review by the Environmental Protection Agency. The primary benefit of the introduction of Bt corn has been increased yields, by 66 million bushels in 1999. Growers have also achieved modest reductions in insecticide use, as only a small proportion of U.S. field corn acreage was sprayed for the target pest prior to the introduction of Bt varieties. The current reregistration process for Bt corn by EPA demonstrates the evolving nature of the regulatory structure for genetically modified crops in response to improved scientific understanding of their risks and benefits.

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