EPA Regulation of Plant-Pesticides and Bt Plant-Pesticide Resistance Management

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Overview of EPA Regulations of Pesticides

The US Environmental Protection Agency (EPA) regulates pesticides under two major statutory authorities: the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), and the Federal Food Drug and Cosmetic Act (FFDCA). Under FIFRA, the EPA has the authority to regulate the development, sale, distribution, use, storage, and disposal of pesticides. To be registered, FIFRA required that a pesticide will not cause "unreasonable adverse effects" to human health or the environment. The Federal Food Quality Protection Act of 1996 (FQPA) modified the test for "unreasonable adverse effects," effective August 3, 1996. The EPA determines if a pesticide would cause an unreasonable adverse effect by considering "the economic, social, and environmental costs [risks] and benefits" of the use of the pesticides. FIFRA generally prohibits the sale or distribution of a pesticide unless it is registered. A product may be registered either unconditionally (see FIFRA section 3(c)5) or conditionally(see FIFRA section 3(c)(7)).

FIFRA, amended by FQPA, defines the term "unreasonable adverse effects on the environment" to mean: "(1) 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, or (2) a human dietary risk from residues that result from a use of a pesticide in or on any food inconsistent with the standard under section 408 of the Federal Food, Drug, and Cosmetic Act" (7 U. S. C. 136(bb)). Before the FQPA amendments took effect on August 3,

*The views expressed in this article are those of the author and do not necessarily represent those of the United States government.

1996, FIFRA contained only the first definition of “unreasonable adverse effects on the environment.” FQPA added the second definition regarding consistency with the FFDCA section 408 standard.

FFDCA gives broad authority to protect human dietary risks that might be posed by the use of any pesticide in food for humans, or as feed for animals. Under FFDCA, the EPA is responsible for determining the amount of pesticide residue that is allowable in raw and processed agricultural commodities” and that may enter commerce. The EPA determines that “there is a reasonable certainty that no harm will result from complete exposure of the pesticide chemical residue, including all anticipated dietary exposures and all other exposures for which there is reliable information”(21 U. S. C. 346a(b)(2) and c(2)(A)).

Types of Pesticides

There are two basic types of pesticides: conventional chemical pesticides and biopesticides. Biopesticides include: microbial, biochemical, and plant-pesticides. Microbial pesticides are living organisms used as pesticides, e. g, microorganisms, fungi, and viruses. Biochemical pesticides are naturally occurring or analogous to naturally occurring pesticidal substances with a non-toxic mode of action against the target pest e. g., pheromones and other semiochemicals used for mating disruption. Plant-pesticides are defined as a pesticidal substance(s) produced in a living plant and the genetic material necessary for the production of that pesticidal substance e. g., delta-endotoxins produced by Cry genes from the soil microorganism *Bacillus thuringiensis* (Bt), expressed in crop plants. These types of plant-pesticides are referred to as Bt plant-pesticides or more commonly as Bt crops.

Regulation of Plant-Pesticides

Regulatory Development

As part of the agreement with the US Department of Agriculture (USDA) and the US Food and Drug Administration (FDA), stated in the Office of Science Technology and Policy’s 1986 Coordinated Framework for Biotechnology Products, the EPA proposed a rule on November 23, 1994 (59 FR 60496, 60519, 60535, 60542, and 60545 Nov. 23, 1994) for the regulation of plant-pesticides. In that proposal, the EPA describes what compounds it considers to be plant-pesticides and how these would be regulated both under FIFRA and FFDCA. In this proposed policy, the EPA makes clear that it would focus its regulatory authority on the pesticidal substances and the genetic material necessary for their production rather than on the plant, *per se*, and designates the pesticidal substances as plant-pesticides. In addition to the policy statement, the EPA issued proposed regulations that define certain categories of plant-pesticides that would be exempt from regulation under FIFRA and FFDCA. Plant-pesticides not exempt would be subject to regulation.

Even though there are no specific plant-pesticide guidelines for data supporting registration, there are regulations governing the registration of these pesticides and requiring the submission of data necessary to enable the Agency to make the requisite findings for registration under section 3(c)(5) and (7). In addition, there are draft guidance documents to aid registrants in their development of appropriate data. After the plant-pesticide rule and regulations are made final, the EPA will issue proposed data requirements for plant-pesticides (including Bt plant-pesticides) and go through a public notice and comment period including holding at least one FIFRA Scientific Advisory Panel meeting.

PROPOSED PLANT-PESTICIDE RULE

The main features of the rule and its status are discussed below.

Definitions

In the proposed policy, plant-pesticides are defined in FIFRA as the pesticidal substance produced in a living plant and the genetic material necessary for the production of that pesticidal substance. This definition is intended to focus the safety assessment on the pesticidal substance itself, rather than the plant in which it was produced. Inclusion of the genetic material as part of the active ingredient of the plant-pesticide recognizes the biological reality that a pesticidal substance will not be produced in a plant without a gene to direct that production. In addition, inclusion of the genetic material provides a mechanism to address the escape of the gene into other plants and a consistent regulatory coverage for parts of a plant's life cycle when the pesticidal substance may not be actively produced.

FIFRA Scope and Exemptions

All plants produce secondary plant compounds that act as pesticidal substances to protect against or mitigate pests. Some plants even produce herbicidal compounds that aid in their colonization of a habitat. The broad definition of plant-pesticide under FIFRA and the extensive knowledge of plant science about certain pesticidal traits suggest that the EPA could easily exempt categories of plant-pesticides from regulation based on a history of exposure and/or safe consumption. A major focus of the rule is to describe these exempt categories of plant-pesticides and explain the triggers for regulatory oversight of other plant-pesticides. The trigger for closer examination under FIFRA focuses on plant-pesticides that have new exposures, either dietary or environmental. Pesticidal traits derived from sources that are not sexually compatible with the host plant would probably not have a history of expression in that host plant and would, as a result, be most likely to cause adverse dietary or environmental effects. Therefore, pesticidal substance originating from a sexually compatible plant species would be exempted from regulation. Also exempted from regulation would be plant-pathogenic virus coat proteins and traits that affect only the host plant such as physical barriers or some types of disease resistance genes.

Labeling

An important feature of the EPA's regulatory approach to plant-pesticides is that it will not register the plant, but rather the plant-pesticide active ingredient and the genetic material necessary for its production. The official FIFRA label is issued to the registrant. There will be no FIFRA-type label accompanying the seed sold in commerce, but rather informational material (referred to here as labeling information) that will instruct the grower on how to use the crop expressing the plant-pesticide (e.g. Bt). The registered label (the FIFRA label) may require that registrants put certain statements or guidance on all informational materials (e.g., technical bulletins, grower guides, Internet materials, videos etc.) that may accompany the Bt crop seed or other propagative material at the time of sale, similar to the information that accompanies seeds treated with conventional pesticides. For example, an informational label statement could tell growers that certain resistance management strategies should or must be followed.

FFDCA Scope and Exemptions

The EPA also has responsibilities under FFDCA to establish a safe level of pesticide residues allowed on food crops. For plant-pesticides, the EPA believes the major human exposure to pesticidal substances will be dietary. There are numerous plant species that have been safely consumed as food. Therefore, plant-pesticides that do not represent a novel dietary exposure in the new host plant would be exempt under the proposed regulations under FFDCA. The triggers for examination under FFDCA are if the pesticidal substance from a normally inedible portion of a food plant is found in the edible portion of the new plant, if the pesticidal substance is from a food plant normally processed before consumption and introduced into a food usually eaten without processing, and if the pesticidal substance has been altered from its original structure or function. The movement of known food allergens from one part of a plant to another part of a plant is being discouraged. The plant-pesticides that qualify for an exemption from the requirement for a food tolerance are those pesticidal substances from sexually compatible plants and viral coat proteins based on the history of safe consumption of these components in the current food supply. In addition, the EPA believes there probably is no dietary risk with the consumption of the small amount of additional genetic material coding for any plant-pesticide so the genetic material necessary for the production of a plant-pesticide is also exempt from a food tolerance.

Current Status

Since the November 1994 publication of the proposed policy in the Federal Register, the EPA has been reviewing the comments received and preparing for publication of the final rule. During the comment review period, Congress passed the Food Quality Protection Act (FQPA) in August 1996 that amended both FIFRA and the FFDCA. These amendments altered some aspects of the process for assessing the food safety of pesticide chemical residues. Therefore, the EPA recently published supplemental notices in the Federal Register to notify the public how the proposed tolerance exemptions for plant-pesticides met the new safety standards of the FFDCA as amended by the FQPA. The final rule for plant-pesticides under FIFRA and associated food tolerance exemptions under FFDCA should be published soon.

Public Meetings Held on Bt plant-pesticides

While there are no published plant-pesticide data guidelines, the Agency has sponsored, or cosponsored with other Federal agencies, four conferences dealing with plant-pesticides and the pertinent data needed to perform a risk assessment: 1) a “Transgenic Plant Conference” in Annapolis, Maryland, September 8-9, 1988; 2) a meeting on “Genetically Engineered Plants: Regulatory Considerations” at the Boyce Thompson Institute for Plant Research, Ithaca, New York, October 19-21, 1987; 3) a conference on “Pesticidal Transgenic Plants: Development, Risk Assessment, and Data Needs”, November 6-7, 1990, and 4) a “Conference on Scientific Issues Related to Potential Allergenicity in Transgenic Food Crops” at Annapolis, Maryland, April 18-19, 1994. In addition, the Agency has requested the advice of four scientific advisory committees on FIFRA and FFDCA-related scientific issues. On December 18, 1992, a Subpanel of the FIFRA Science Advisory Panel (SAP) was convened to review a draft policy statement on plant-pesticides and respond to a series of scientific questions posed by the EPA’s approach under FIFRA. On July 13, 1993, a Subcommittee of the EPA Biotechnology Science Advisory Committee (BSAC) was convened to address a series of scientific questions primarily on the EPA’s approach under FFDCA. On January 21, 1994, a joint meeting of the SAP/BSAC Subpanel on plant-pesticides was held to discuss additional scientific questions. Information from these conferences and scientific advisory committees has been used by the Agency to develop a “points to consider” document as guidance for what data are required to support the registration of plant-pesticides and development of the draft plant-pesticide policy and regulations. The Agency has also provided guidance to registrants on the elements needed for a resistance management strategy for plant-pesticides. On March 1, 1995, a Subpanel of the FIFRA SAP was convened to review the Agency’s risk assessment and resistance management analysis for Bt potato (CryIIIA).

Two independent Subpanels of the FIFRA SAP met, in part, to address resistance management of Bt crops. On December 18, 1992, a Subpanel of the FIFRA SAP addressed the issue of development of pest resistance to pesticidal substances produced by plants. The Subpanel felt that delaying the evolution of resistance was very important and urged the EPA to actively assess the problem of pesticide resistance, especially when the pesticide is part of the progression toward use of "safer" pesticides. A third independent SAP Subpanel met exclusively to discuss Bt plant-pesticide resistance management issues on February 9-10, 1998. The findings of this Subpanel are discussed below.

The March 1, 1995 SAP met to discuss in part, resistance management of Bt crops. This subpanel was in agreement with the Agency's review of the Monsanto plan for Bt CryIII(A) delta-endotoxin produced in potatoes and the general elements necessary for a resistance management plan to address resistance to Bt delta-endotoxins produced in potatoes. The Agency and the SAP agreed that Monsanto's resistance management strategy for the potato variety producing the Bt CryIII(A) delta endotoxin, although adequate for the present, should be further refined in the future as additional data become available. Many of the specific questions with respect to monitoring for resistance development and strategies to retard resistance development can best be addressed when use of the potatoes producing the Bt CryIII(A) delta endotoxin has reached commercial scale production over a period of several years throughout potato producing regions. Refinements of resistance management strategies are typically needed during the years of actual use of any pesticide. Monsanto agreed to voluntarily implement the resistance management plan for the Bt CryIII(A) delta endotoxin produced in potatoes and has agreed to continue to voluntarily work with the Agency on refinements to the resistance management plan as more information is gathered during wide-scale commercial use.

Although the 1995 SAP meeting focused primarily on the review of the risk assessment and resistance management issues for Bt potatoes, the FIFRA SAP Subpanel also generally discussed resistant management issues for Bt corn and Bt cotton. The Subpanel members recommended that in order to refine existing resistance management plans, large-scale use of these plant-pesticides was needed. The Agency agreed with this approach and is allowing such large-scale use, with appropriate safeguards to protect against the development of resistance, while requiring registrants to conduct research necessary to develop acceptable long-term resistance management plans.

The Agency has raised, in general, the issue of pesticide resistance management to its Pesticide Program Dialogue Committee (PPDC) in July 1996. The PPDC supports the EPA's continued efforts to protect the use of Bt foliar pesticides and plant-pesticides. The EPA has also held two public hearings, one on March 21, 1997 (in Washington D. C.) and the other on May 21, 1997 (College Station, Texas), to solicit comments on resistance management plans for plant pesticides. There were four issues open for comment: 1)the requirement for resistance management plans, 2) scientific needs for resistance management plans, 3) use of "public good" as a criterion for the requirement of resistance management plans, and 4) 1996 performance of Bt cotton.

Approximately 100 individuals/organizations submitted written comments and/or delivered presentations regarding the subject of Bt plant-pesticide resistance management and the four issues open for comment. Copies of the written comments are available in the Office of Pesticide Programs public docket, OPP-00470.

The EPA held an Office of Pesticide Programs FIFRA SAP meeting on February 9-10, 1998 to examine the resistance management strategies for Bt delta-endotoxins expressed in potatoes, field corn, and cotton. The Agency published a recent analysis of the current resistance management strategies for Bt potato, Bt field corn, and Bt cotton in a paper entitled "The Environmental Protection Agency's White Paper on Bt Plant-Pesticide Resistance Management" (January 14, 1998)-(EPA, 1998a). In this paper, the Agency summarized the findings from the March and May 1997 public hearings on Bt plant-pesticide resistance management (OPP Docket, OPP-00470), the 1996 growing season reports on resistance management activities for Bt potato, Bt field corn, and Bt cotton, and 1997 research efforts for resistance management strategies (EPA, 1998a). The Agency asked the SAP Subpanel to review specific questions posed by the EPA based on its "White Paper. "Oral and written statements were received from approximately 20 different groups representing industry, growers or grower groups, trade organizations, academia, and environmental groups. The Subpanel provided the Agency with a final report of the meeting on April 28, 1998 (SAP, 1998). Copies of the written statements and the Subpanel report can be obtained from the OPP Docket Office (OPPTS-00231). The EPA White Paper can also be obtained electronically from the EPA Home Page at: Federal Register—Environmental Documents—"Laws and Regulations" (http: //www. epa. gov/fedrgstr/). A summary of the key points made in the Subpanel report and in the White Paper will be discussed later in this article. Other SAP meetings are planned on Bt plant-pesticide resistance management in the next several years.

Registration of Bt Plant-pesticides: Scientific Data Considerations

Each registered Bt plant-pesticide has undergone a determination that the proposed use of the plant-pesticide poses no "unreasonable adverse effects", including a thorough review of the human health and environmental risks and a benefit assessment. Under FIFRA section 3(c)(7), time-limited conditional registrations have been registered during which time the company is addressing questions that were unanswered at the time of initial application. There have been two types of plant-pesticides approved by the EPA to date: delta-endotoxins derived from Bt and coat proteins from plant pathogenic viruses. In addition, five other genes termed marker genes, used to tag the desired trait during the trait development process and carried along with the plant pesticide genes, have been evaluated by the EPA for food safety. These products have had safety assessments done by the EPA for both human health and environmental effects. The basis of the assessment was an accurate characterization of the newly introduced trait, a description of the host plant biology, and adequate information to assess the toxicity of the expressed pesticidal compound to humans and exposed non-target species. A summary of all of the science review findings and regulatory management conclusions for each of the registered Bt plant-pesticides is found in the EPA Pesticide FACT sheets (EPA 1995a, b, c, 1996a, b, 1997, 1998b, c, d).

Characterization of the Active Ingredient

Fundamental to the EPA's risk assessment of the Bt plant-pesticides was a thorough description of these plant-pesticides including the source of the inserted sequences necessary to produce the pesticidal substance and any novel proteins encoded by this introduced genetic material. For the individual delta-endotoxins, a great deal of historical information was available to the EPA due to the numerous registered microbial products known to contain such endotoxins. However, the companies were required to verify that the inserted DNA did, in fact, code for the toxins claimed and that these plant-expressed toxins were similar to those found in the microbial products. This similarity analysis was done using standard protein biochemistry analyses such as amino acid sequencing, immunological recognition as well as biological activity against target pests. Additionally, the expression of the pesticidal substance was determined for various tissues at different maturities. Since the pesticidal substances and associated proteins were adequately characterized, a reasonable prediction of the type of data necessary to evaluate potential risks for mammalian and environmental effects was proposed.

Human Health Risk Assessment

Dietary consumption was determined to be the predominant route of exposure to humans and domestic animals for the crops engineered to express these pesticidal substances. For crops producing proteinaceous pesticidal substances, mammalian toxicology was assessed by acute oral studies in the rodent. If significant prior human dietary exposure to the plant-pesticide could be documented, some acute mammalian toxicology studies were waived. When required, these acute oral studies in rodents were done with high doses of a purified test material such as 2-5gm/kg bodyweight. No abnormalities were seen in any tests done with the plant-pesticidal substances or related compounds examined to date. The EPA also assessed information provided to indicate the introduced traits were not responsible for a food allergy. This information included a screen for amino acid homology to known food allergens and an in vitro digestibility assay in artificial digestive fluids to address the potential for a protein to persist in dietary exposure and possibly induce food allergy or other toxicity. For all the pesticidal traits seen to date, the lack of mammalian toxicity has justified an exemption from the requirement for a food tolerance as required by the EPA's responsibility under FFDCA.

Environmental Fate and Ecological Risk Assessment

Ecological nontarget data needs are driven by exposure to the plant-pesticide. The pesticidal active ingredient (e. g., the Bt delta-endotoxin and the genetic material necessary for its production) is contained only within certain plant parts of the crop plant into which it has been genetically engineered. This means that nontarget organisms will only have a minimal exposure to the pesticidal active ingredient. This type of exposure situation is quite different from that associated with spray applications of pesticides. Exposure of nontarget organisms to plant pesticides would occur primarily when wildlife feed on plants expressing the pesticidal substance or if sexual transfer of the new trait(s) to nontarget wild/weedy relatives occurs by cross-pollination.

Therefore, the ecological effects data are based on the expected exposure of non-target species to the plant-pesticide and by geographical use considerations based on the proximity to related cultivars or weedy relatives that can cross-pollinate with plants expressing the pesticidal substance. This amounts to a case-by-case analysis. Each risk assessment is made from an analysis of the properties of the engineered organism and its target environment, i.e. on the nature of the gene being introduced, the plant receiving the gene, the environment where the plant will be grown, and the species susceptible to the effects of the introduced gene. The degree of scrutiny depends on the type of gene product, i.e. the protein or the product of metabolic pathway more akin to conventional chemicals, and the intended mode of action. Protein products are not expected to pose much, if any, nontarget hazard outside of living plant tissue, while chemical compounds may be more resistant to degradation, more toxic, and have a broader exposure.

For environmental effects, the EPA has examined the toxicity of the plant-pesticidal traits in plant tissue to non-target organisms. The specific non-target organisms tested were chosen as indicators of potential environmental effects and are similar to those examined for microbial or biochemical pesticides. The choice of appropriate indicator organisms for testing was based on the potential exposure from data on plant-pesticide expression in the engineered plant. Trait expression data are used to predict exposures for target organisms that may impinge on resistance management decisions. For Bt plant-pesticides, the EPA has examined the toxicity of the pesticidal substance to birds, fish, honeybees and certain other beneficial insects. Among the beneficial species, data on Collembola and earthworm species may be required if crop residue exposure is a possibility. In the honeybee study, effect studies on immature individuals as well as adults may be required if exposure to the Bt delta-endotoxin in pollen is expected. The Agency has examined the environmental fate endpoints regarding the movement and expression of the gene trait in other plant species (biological fate) and persistence of the pesticidal product in the environment (chemical fate). Specifically the environmental fate endpoints are: a) gene product (chemical) persistence and movement in the environment, b) potential for the genetically engineered plant to survive outside of cultivation and become a weed (i.e. weediness potential), and c) potential for the introduced genetic trait to confer a selective advantage to a wild relative (i.e. outcrossing potential and ecosystem disruption). Data on the toxicity of the gene product to nontarget insects are required when the proposed use pattern indicates that insect predators and/or parasites may be exposed to the pesticide. Appropriate test species should be chosen based on the ecosystem where the plant-pesticide will be used.

THE EPA'S REVIEW OF BT-PLANT-PESTICIDE RESISTANCE MANAGEMENT

With a greater focus on pollution prevention and pesticide risk reduction, the EPA believes that it is important to implement effective resistance management strategies for pesticides such as Bt plant-pesticides. Bt plant-pesticides and Bt microbial pesticides are recognized as safer pest control resources and are part of the "public good."A great deal of Agency attention has focused on the potential development of resistance to the delta-endotoxins of Bt genetically-engineered into plants (Bt plant-pesticides). This is because Bt plant-pesticides produce the pesticidal active ingredient, the Bt delta-endotoxin(s), throughout the growing season. Long-term exposure to a pesticide is one of the factors that increases the potential selection pressures upon both the target pests and any other susceptible insects feeding on the transformed crop. The EPA recognizes the value of Bt plant-pesticides as effective and safer pest management tools and has determined it is appropriate to conserve this resource by requiring resistance management plans for certain transformed crops. In addition to Bt delta-endotoxins being used in plant-pesticides, they are also widely used in a variety of Bt microbial spray products on many crops. Because the high benefits of using Bt plant-pesticides could be diminished by the development of resistance to individual Bt plant-pesticides and the threat cross-resistance poses to Bt microbial pesticides, the Agency has requested that all registrants for Bt plant-pesticides voluntarily submit pesticide resistance management strategies as part of the registration submission.

The Agency identified seven elements that should be addressed in a Bt plant-pesticide resistance management plan (Matten and Lewis, 1995). These elements are: 1) knowledge of pest biology and ecology, 2) appropriate dose expression strategy, 3) appropriate refugia (primarily for insecticides), 4) monitoring and reporting of incidents of pesticide resistance development, 5) employment of IPM, 6) communication and educational strategies on use of the product, and 7) development of alternative modes of action. These elements were presented to the March 1, 1995 Federal Insecticide, Fungicide, and

Rodenticide Act (FIFRA) Science Advisory Panel (SAP) Subpanel on Plant-Pesticides. The SAP Subpanel approved of these seven factors (SAP, 1995; see Office of Pesticide Program (OPP) docket, OPP-00401). These elements are discussed in more detail in Matten et al. (1996) and EPA White Paper on Bt plant-pesticide resistance management (1998a). All registrants of Bt plant-pesticides voluntarily submitted Bt plant-pesticide insect resistance management strategies to the Agency for Bt delta-endotoxins produced in potato (Bt potato); field corn (Bt corn), sweet corn (Bt sweet corn), and popcorn (Bt popcorn); and cotton (Bt cotton). When necessary, the Agency made certain recommendations and requirements of registration for data to develop and implement long-term resistance management strategies as part of the registration decisions. The Agency's reviews of the resistance management strategies for registered Bt plant-pesticides are summarized in the EPA Pesticide FACT sheets (EPA 1995a, b, c, 1996 a, b, 1997, 1998 b, c, d).

In May 1995, the Agency registered the CryIIIA delta-endotoxin and the genetic material necessary for its production in potato (Bt potato). Following the advice of the March 1, 1995 SAP Subpanel, a resistance management plan for Monsanto/Naturemark's Bt potato was voluntary rather than mandatory. EPA and Monsanto/Naturemark have worked together on the development and implementation of appropriate long-term resistance management following the registration of Bt potatoes in 1995. Monsanto/Naturemark requires growers to sign a Grower Agreement to use the technology. The Grower Agreement specifies that each grower must follow a mandatory structured refuge. The original Bt potato resistance management strategy has been refined as more data became available.

The Agency mandated specific resistance management data requirements and mitigation measures with a resistance management strategy for all of the Bt corn and Bt cotton registrations. Registrations for Bt corn plant-pesticide products expire April 1, 2001 and the registration for Bt cotton plant-pesticide products expire January 1, 2001. These registrations were conditional to allow, in part, for completion of the studies related to resistance management. Collection of various data, e. g. , target pest biology and behavior, secondary pest biology and behavior, population dynamics, cross-resistance potential, refuge strategies, dose deployment adequacy, baseline susceptibility, discriminating concentration, monitoring, and reporting were made conditions of registration for the Bt corn and Bt cotton registrations. Refuge requirements were mandatory for Bt cotton. Development of draft refuge options by August 1998, a final refuges strategy by January 1999 with implementation by April 1, 2001were required of Bt corn registrations. As part of the terms and conditions of registration, the EPA will reevaluate the effectiveness of each registrant's resistance management plan before the expiration date and decide on whether to convert the registration to a non-expiring registration.

The Agency registered the use of CryIA(b) in sweet corn (Bt sweet corn) and popcorn (Bt popcorn) in March 1998. Specific monitoring and sales reporting were made requirements of the Bt sweet corn registration. No specific refuge requirements were mandated for Bt sweet corn (Event BT 11) because harvesting occurs before insects mature, approximately 21 days after silking. Growers are instructed in all labeling and technical material to destroy any CryIA(b) sweet corn silks that remain in the fields following harvest or within a short period of time (a maximum of one month) later in accordance with local production practices. Stalk destruction will help reduce the possibility of larvae surviving to the next generation. The Bt sweet corn registration expires April 1, 2001. The Agency mandated specific refuge requirements on the use of Bt popcorn (Event 176) based on the USDA NC-205 recommendations (Ostlie et al. , 1997). Specifically, a 20-30 percent unsprayed or 40 percent sprayed non-Bt corn structured refuge in close proximity to Bt corn is required. Spraying with pesticides reduces the effectiveness of the refuge. The refuge must be established within 0.5 miles of the Bt corn. Specific monitoring and sales reporting requirements were also made for the Bt popcorn registration.
All previous data required for Bt field corn were also required for Bt popcorn. The Bt (Event 176) field corn and popcorn registrations expire April 1, 2001.

The Agency registered the use of Cry9(c) field corn in May, 1998. This is a one-year registration for 120,000 acres for animal feed, industrial non-food, and seed increase uses expiring on May 30, 1999. EPA mandated specific refuge requirements based on the USDA NC-205 recommendations (Ostlie et al., 1997). Specifically, a 25 percent unsprayed or 40 percent sprayed non-Bt corn structured refuge must be planted within 1500-2000 feet of Bt corn. Because of the one-year duration of this registration, only sales reporting and grower education are required as part of this registration. Additional resistance management factors must be addressed for a full commercial registration.

All stakeholders are concerned with how the EPA regulates resistance management for Bt plant-pesticides. Scientifically sound long-term resistance management strategies are essential to the survival of Bt plant-pesticides, maintaining the effectiveness of Bt microbial pesticides, and reduction in the risks from the use of chemical pesticides. The EPA is continuing to evaluate and refine how it regulates resistance management of Bt plant-pesticides. The EPA has worked and is working with stakeholders (industry, extension and research entomologists and other academic scientists, USDA, individual growers, user groups, trade organization, public interest groups, and government agencies) to address long-term resistance management strategies for Bt plant-pesticides.

THE EPA WHITE PAPER ON BT PLANT-PESTICIDE RESISTANCE MANAGEMENT

As noted earlier, the EPA published a recent analysis of the current resistance management strategies for Bt potato, Bt field corn, and Bt cotton in a paper

TABLE 1. REGISTERED BT PLANT-PESTICIDES AND ACTIONS PENDING

Events/ Products	Year Registered	Expiration Date	Toxin	Crop	Company(s)
New Leaf®	May 1995	None	Cry IIIA	Potato	NaturMark/ Monsanto
Bollgard™	October 1995	January 2001	Cry IA (c)	Cotton	Monsanto
Event 176	August 1995	April 2001	Cry I(A)b	Field Corn	Novartis Seeds and Mycogen Corporation
Event 176	March 1998	April 2001	Cry I(A)b	Popcorn	Novartis Seeds
BT11	October 1996	April 2001	Cry I(A)b	Field Corn	Novartis Seeds
BT11	March 1998	April 2001	Cry I(A)b	Sweet Corn	Novartis Seeds
MON810	December 1996	April 2001	Cry I(A)b	Field Corn	Monsanto
DBT-418	March 1997	April 2001	Cry IA (c)	Field Corn	DeKalb Genetics Corporation
CBH-351	May 1998	May 1999	Cry 9(c)	Field Corn	AgrEvo/PGS

entitled "The Environmental Protection Agency's White Paper on Bt Plant-Pesticide Resistance Management" (EPA, 1998a). A summary of the EPA's White Paper is provided below.

WHITE PAPER SUMMARY

Since Bt plant-pesticides became commercially available in 1996, growers have adopted this technology as part of their Integrated Pest Management (IPM) practices to control pests in potato, corn, and cotton. Based on industry reports sent to the EPA, the greatest adoption of Bt crop technology has been by cotton growers, especially in the southeastern United States in 1996, with about 13 percent of the cotton acreage, 1. 8 million acres, and an estimated 2.2 to 2.4 million acres in 1997 planted in Bt cotton. Corn growers planted about 400,000 acres of Bt corn in 30 states in 1996 and an estimated four million acres in

1997. Potato growers planted about 10,000 acres of Bt potato in 1996 and an estimated 25,000 acres in 1997. The differences in the rate of adoption of Bt potato, Bt corn, and Bt cotton are likely due, in part, to the availability of effective alternatives, the cost of the biotechnology crop, extent of regional pest problems, and familiarity and acceptance of the technology by growers. For example, there are several insecticide alternatives for control of Colorado potato beetle. The cost of and lack of familiarity with the technology and type of hybrids available may have discouraged a wider adoption by corn growers in the first years of commercialization. The adoption rate for Bt cotton was especially high for a new technology because few, if any, effective alternatives existed to control tobacco budworm (*Heliothis virescens* (Fabricius), TBW) in cotton especially where insect resistance to registered conventional pesticides was extremely high in states such as Mississippi and Alabama.

No evidence exists that resistance to Bt delta-endotoxins expressed in transgenic potato, corn, or cotton has developed in the 1996 or 1997 growing season. Monitoring for changes in susceptibility to the different registered Cry proteins, CryI(A)b, CryI(A)c, and CryIIIA, has been conducted for TBW, Colorado potato beetle (Leptinotarsa decemlineata (Say), CPB), European corn borer (*Orsinia nubilalis* (Habner), ECB), cotton bollworm (*Helicoverpa zea* (Boddie), CBW), and pink bollworm (*Pectinophora gossypiella* (Saunders), PBW). Baseline susceptibility studies show a wide-range of variability, so it is important to look at susceptibility changes in the context of the baseline range for a particular geographic location of the pest (i. e., different portions of a state). No changes in baseline susceptibility have been detected for any of the target insects exposed to the Bt delta-endotoxins expressed in Bt potato, Bt corn, and Bt cotton. This information indicates that there has been no measured increase in tolerance to date to the Bt delta-endotoxins expressed in potato, field corn, and cotton.

Toxin-tolerant colonies of CPB, ECB, TBW, CBW, and PBW have been created in the laboratory through selection against purified Cry proteins or mixtures of Cry proteins using Bt microbial pesticides. The ability of insects to develop high levels of tolerance to Bt in the laboratory indicates that these insects possess the genetic potential to develop resistance to Cry delta-endotoxins expressed as Bt plant-pesticides. Laboratory-tolerant colonies are useful to study the genetics and biochemistry of resistance of possible resistance mechanisms that may exist in the field. It is unlikely that laboratory selective procedures provide the identical selective conditions that exist in the field. The ability to select for tolerance to Cry proteins in the laboratory in different insect pests indicates that it is prudent to use appropriate resistance management strategies.

In 1996, cotton bollworm populations were the highest seen in ten years in parts of the Cotton belt (i. e., Brazos Valley, Texas, Mid-South and Southeast growing regions). Monsanto reported to the Agency the potential Bt cotton control failures as early as July, 1996, and followed up with a full analysis of these incidents in the fall of 1996. Monsanto performed studies in all Bt cotton areas affected by high CBW infestations to determine whether CBW susceptibility to the CryI(A)c toxin had changed, whether the Bt cotton was expressing the CryI(A)c, and whether the CryI(A)c expression levels and patterns had changed. Monsanto also provided the results of these studies in its 1996 annual report on resistance monitoring activities. Results of these studies indicate that there was no change in CBW susceptibility and no change in Bt expression in the Bt cotton areas affected by high cotton bollworm infestations. These studies indicated no detectable level of resistance in these populations. Unusually high infestation levels of CBW may have, in part, resulted from the dramatic increase in corn acreage in the South. In addition, CBW has a lower sensitivity (about 25-fold lower) to the CryI(A)c delta-endotoxin relative to TBW and PBW. Scouting detected CBW larvae lower in the plant canopy of Bt cotton than expected and, in some cases, supplemental chemical insecticides were used to control CBW. The fact that supplemental insecticides might be necessary to control unusually high CBW infestations was not unexpected and was considered in the Agency's review of the initial resistance management strategy for Bt cotton. Modifications to the CBW scouting program for Bt cotton were made for the 1997 season to improve detection of the CBW larvae which might escape the Bt delta-endotoxin by feeding on blooms and bloom tags that are lower in the cotton plant.

The vast majority of cotton growers complied with the structured refuge requirements. Cotton growers seem to prefer the 20 percent sprayed refuge option that allows them to treat the refuge with chemical insecticides normally used to control TBW, CBW, and PBW (except for Bt microbial pesticides). This option appears to provide a higher yield in the refuge acreage than the four percent unsprayed refuge option that often had higher management costs and lower yields. Most cotton researchers, who commented at the two public hearings held in March and May 1997, favored the 20 percent structured refuge as a better strategy for Bt cotton resistance management. They believed that this refuge option is more likely to provide a greater percentage of susceptible insects throughout the growing season to mate with any rare resistant individuals that might survive in the Bt cotton fields. The EPA received comments that the four percent unsprayed refuge was decimated early in the growing season so that there were few, if any, adult moths surviving to mate with any resistant insects that survived in the Bt cotton fields later in the growing season. The EPA believed that during the first five years following the first complete growing season in 1996, there would not be enough Bt corn acreage to provide substantial Bt selection pressure for the development of ECB resistance. Consequently, the EPA did not mandate specific refuge requirements for Bt corn, but the EPA has required research data on the size, structure, and deployment of a structured refuge. A combination of temporal and structured refuges is being studied. A draft refuge strategy must be submitted to the

Agency by August, 1998, and a final refuge strategy is required to be submitted by January, 1999. Implementation of an EPA-approved structured refuge plan or an EPA-approved alternative resistance plan is required no later than April 1, 2001. Monsanto and Dekalb are requiring structured refuges, either a five percent unsprayed or 20 percent sprayed structured refuge, as part of grower agreements. Beginning in the 1998 growing season, Novartis Seeds has adopted the NC-205 consortium's recommendations published in NCR-602 publication entitled "Bt Corn & European Corn Borer - Long Term Success Through Resistance Management" (Ostlie et al., 1997). As noted earlier, the NC-205 recommended a 20-30 percent structured non-Bt corn refuge to prevent Bt delta-endotoxin exposure to 20-30 percent of the larval populations. They also recommended that in continuous corn acreage sprayed with insecticides, the refuge size would be increased to 40 percent to compensate for larval mortality. In addition, a smaller refuge size may also be suitable if there are many alternate hosts providing adequate numbers of susceptible ECB. Mycogen has not made any specific refuge recommendations in its Grower Guide, but is supportive of the use of refuges and supportive of the NC-205 recommendations.

Monsanto/Naturemark requires a structured refuge as part of grower agreements for use of Bt potato. The EPA has required that Monsanto mandate specific refuge requirements as a condition of registration for Bt cotton, either a four- percent unsprayed or 20 percent sprayed structured refuge. Monsanto has implemented these refuge requirements through a grower agreement. Research is underway to study whether in-field narrow strip refuges or mixed Bt cotton/non-Bt cotton seed mix options are viable for PBW resistance management because of the limited larval movement. Based on Monsanto's reports to the Agency, there has been a high level of compliance with a structured refuge in Bt cotton and Bt potato. The EPA is encouraged by reports of a tremendous reduction in the use of conventional insecticides that has resulted from adoption of Bt cotton (i.e., 250 thousand gallons of formulated product).

A great deal of research is underway to study the elements that are necessary for long- term resistance management strategies for Bt potato, Bt corn, and Bt cotton. Specific research data were required as part of the Bt corn and Bt cotton conditional registrations and was recommended for the Bt potato registration. These data included: the dosage effectiveness on the target pest(s), monitoring data including baseline susceptibility and validation of the diagnostic dose concentration, pest biology and ecology, influence of the Bt crop on secondary lepidopteran pests, the impact of CryIA(b)/CryIA(c) produced in Bt corn on the selection of CEW/CBW resistance in Bt corn and Bt cotton, impact of Bt on CEW overwintering survival and fecundity, effective refuges, alternate hosts as refuges, and cross-resistance potential. Additionally, alternative pest control strategies and integration into existing IPM programs are being examined for each of the Bt plant-pesticides. All of these data will provide the basis for specific improvements to the existing resistance management strategies. Future information is especially important for understanding the selection of CEW/CBW resistance in overlapping Bt corn and Bt cotton regions of the southern United States. This is because CEW/CBW usually moves from silking corn to cotton, has multiple generations per year, and overwinters in the South. Exposure to Cry delta-endotoxins produced in both Bt corn and Bt cotton in two or more generations per year could rapidly accelerate development of resistance. Research results and predictive models studying this situation are expected to be submitted to the Agency in 1998.

Science Advisory Panel Review of EPA's White Paper

The Agency asked the February 9-10, 1998 OPP FIFRA Science Advisory Panel Subpanel on Bt plant-pesticide resistance management to review specific questions posed by the EPA based on its "White Paper" (EPA, 1998a) on Bt plant-pesticide resistance management strategies for Bt potato, Bt corn, and Bt cotton. Oral and written statements were received from approximately 20 different groups representing industry, growers or grower groups, trade organizations, academia, and environmental groups. The Subpanel provided the Agency with a final report of the meeting on April 28, 1998 (SAP, 1998). Copies of the written statements and the Subpanel report can be obtained from the OPP Docket Office (OPPTS-00231). The Subpanel's report can also be obtained electronically. A brief summary of key points made in the Subpanel report is provided below. The Subpanel agreed with the EPA that the wide-spread use of crops that express Bt insecticides is in the public good by providing additional pest control options to producers and by reducing the use of conventional pesticides. The Subpanel also agreed with the EPA that appropriate resistance management is necessary to suppress the emergence of insect resistant to Bt toxins expressed in transgenic crop plants. The Subpanel recognized that resistance management programs should be based on the use of both high dose expression levels and structured refuges designed to provide sufficient numbers of susceptible adult insects with a minimum of economic impact on producers. Resistance management strategies should be sustainable and to the extent possible, strongly consider grower acceptable and logistical feasibility. The Subpanel made the following overall recommendations and conclusions: a) EPA should require the use of structured refuges in all registrations of Bt crops (unless proven to be harmful), b) a refuge/high dose strategy is needed to delay the development of resistance for targeted pests, c) precision of research models is good for evaluating refuge options, but is limited in establishing specific refuge options, d) the EPA should establish regional working groups for specific implementation of resistance management strategies for each of the major Bt crop producing regions, e)grower participation is the key factor for successful implementation of a resistance management strategy, and f) regulatory strategies should serve growers with a sustainable approach to resistance management that encourages compliance with a resistance management strategies. The Subpanel defined a high dose as 25 times the amount of Bt delta-endotoxin necessary to kill susceptible individuals. A cultivar could be considered to provide a high dose if verified by at least two of the following five approaches: 1) Serial dilution bioassay with artificial diet containing lyophilized tissues of Bt plants using tissues from non-Bt plants as controls; 2) bioassays using plant lines with expression levels approximately 25-fold lower than the commercial cultivar determined by quantitative ELISA or some more reliable technique; 3) survey large numbers of commercial plants in the field to make sure that the cultivar is at the LD99.9 or higher to assure that 95 percent of heterozygotes would be killed (see Andow and Hutchison, 1998); 4) similar to (3) above, but would use controlled infestation with a laboratory strain of the pest that had an LD50 value similar to field strains; and 5) determine if a later larval instar of the targeted pest could be found with an LD50 that was about 25-fold higher than that of the neonate larvae. If so, the stage could be tested on the Bt crop plants to determine if 95 percent or more of the later stage larvae were killed.

The Subpanel defined structured refuges to “include all suitable non-Bt host plants for a targeted pest that are planted and managed by people. These refuges could be planted to offer refuges at the same time when the Bt crops are available to the pests or at times when the Bt crops are not available. “The Subpanel stated that a good resistance management strategy should provide efficacy of the toxin(s) for more than 10 years. The Subpanel suggested that a production of 500 susceptible adults in the refuge that move into the transgenic fields for every adult in the transgenic crop area (assuming a resistance allele frequency of 5 $X10^{-2}$) would be a suitable goal. The placement and size of the structured refuge employed should be based on the current understanding of the pest biology data and the technology.

MONITORING FOR BT PLANT-PESTICIDE RESISTANCE

The EPA currently mandates that both baseline susceptibility and a discriminating concentration be developed for each labeled target pest for Bt corn and Bt cotton registrations (see EPA 1995b, c, 1996a, 1996b, 1997, 1998b, c, d).

Monsanto/Naturemark voluntarily instituted a monitoring program for Bt potato. If a discriminating concentration assay is unavailable then the registrant must proceed with efforts to develop discriminating concentrations assays for these target pests and ensure that monitoring studies are conducted annually to determine the susceptibility of all the labeled target pests to the Bt plant-pesticide. The resistance-monitoring program is being developed to measure increased tolerance to Bt plant-pesticides above regional/state/local baseline ranges. The results of the baseline susceptibility and monitoring studies must be communicated to the Agency on an annual basis, by January 31 of the year following the population collections for a given growing season. These annual reports must also describe progress towards development of a discriminating dose assay for each target pest. These current requirements provide the Agency with standardized information to determine whether resistance evolution is occurring. However, there are additional monitoring techniques, other than the discriminating concentration assay, which may be more aggressive to proactively determine whether resistance is developing such as a F2 screen (Andow and Alstad, 1998, Andow et al., 1998), in-field surveys including sentinel plots, screening against test stocks (see Gould et al., 1997). The SAP Subpanel report (SAP, 1998) provides a more detailed discussion of available monitoring techniques.

NEXT STEPS

The EPA is reviewing the Subpanel report and other materials submitted as a result of the February 9-10, 1998 SAP Subpanel Meeting. This information will contribute to how EPA continues to evaluate and refine its regulation of resistance management for Bt plant-pesticides.

The EPA will continue to work with stakeholders from industry, Extension and research entomologists and other academic scientists, user groups, trade organizations, public interest groups, and government agencies to address long-term resistance management for Bt plant-pesticides.

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