EPA REGULATION OF TRANSGENIC PESTICIDAL CROPS AND INSECT RESISTANCE MANAGEMENT FOR B.T. COTTON

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Abstract

The U.S. Environmental Protection Agency (EPA) regulates transgenic pesticidal crops. EPA believes that scientifically-sound long-term insect resistance management strategies are essential to the survival of *Bacillus thuringiensis* (*B.t.*) plant-pesticides, protection of *B.t.* microbial pesticides, and reduction in the risks from the use of pesticides. EPA imposed mandatory insect resistance management requirements for *B.t.* cotton. Two structured refuge requirements were imposed: 4% unsprayed or 20% sprayed Work is underway to reevaluate all of the existing IRM plans for *B.t.* crops (*B.t.* corn and *B.t.* cotton) prior to their 2001 expiration dates. The Cry1Ac cotton registration expires January 1, 2001. EPA will have a public process in 2000 to reevaluate the current IRM plans prior to making regulatory decisions for the 2001 growing season. EPA will continue to work with stakeholders from industry, academia, trade organizations, public interest groups, and government agencies to address long-term insect resistance management for *B.t.* crops.

Overview of EPA's Regulation of Pesticides

The US. Environmental Protection Agency (EPA) regulates pesticides under two statutory authorities: the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and the Federal Food Drug and Cosmetic Act (FFDCA). The Food Quality Protection Act of 1996 (FQPA) amended both FIFRA and FFDCA to establish a uniform standard for pesticide residues in food. Under FIFRA, EPA has the authority to regulate the development, sale, distribution, use, storage, and disposal of pesticides. To be registered, FIFRA requires that a pesticide will not cause "unreasonable adverse effects" to human health or the environment. 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.

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, EPA is responsible for determining the amount of pesticide residue or tolerance that is allowable in raw and processed agricultural commodities and that may enter commerce. Under FQPA, EPA must determine whether "there is a reasonable certainty that no harm with result from aggregate exposure of the pesticide chemical residue, including all anticipated dietary exposures and all other exposures for which there is reliable information."

Types of Pesticides

There are three basic types of pesticides: synthetic chemical pesticides, antimicrobial 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 pesticidal substance(s) produced in a living plant and the genetic material necessary for the production of that pesticidal substance. For example, delta-endotoxins produced by *cry* genes from the soil microorganism, *Bacillus thuringiensis* (*B.t.*), expressed in potato, corn, and cotton would be considered *B.t.* plant-pesticides, or more commonly as *B.t.* crops.

Regulation of Plant-Pesticides

Regulatory Development

As part of the agreement with the United States Department of Agriculture and the Food and Drug Administration stated in the Office of Science Technology and Policy's 1986 Coordinated Framework for Biotechnology Products, 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 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 Agency made 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 designated the pesticidal substances as plant-pesticides. In addition to the policy statement, the Agency 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 plant-pesticide specific guidelines for data supporting registration, there are regulations governing the registration of all pesticides, including plant-pesticides, and requiring the submission of data necessary to enable the Agency to make the necessary regulatory decisions. In addition, there are draft guidance documents to

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aid applicants in their development of appropriate data. The Agency has conducted a number of public meetings regarding the science and policy issues for plant-pesticides in general and *B.t.* plant-pesticides in particular. These were held both prior and subsequent to the proposal of the plant-pesticide rules. The Agency has held six Office of Pesticide Program (OPP) FIFRA Scientific Advisory Panel (SAP) Meetings, two Pesticide Program Dialogue Committee (PPDC) meetings, and two EPA public hearings. The Agency has also sponsored, or cosponsored with other Federal agencies, four conferences dealing with plant-pesticides and the pertinent data needed to perform a risk assessment. In 1997, EPA held one workshop on general plant-pesticide issues and in 1999, EPA held two workshops on insect resistance management for *B.t.* crops. After the plant-pesticide rule and regulations are made final, EPA will propose data requirements for plant-pesticides including *B.t.* plant-pesticides, and go through a public notice and comment period. Both the proposed and final data requirements will be peer-revived at FIFRA Scientific Advisory Panel meetings. EPA held one SAP meeting, December 8, 1999, on the development of product characterization and non-target data requirements. Other SAP meetings are planned for the year 2000.

Labeling

An important feature of EPA's regulatory approach to plant-pesticides is that the Agency is not regulating the whole 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. Informational material instructing producers on how to use the crop expressing the *B.t.* plant-pesticide will accompany seeds sold in commerce. The registered label (the FIFRA label) will require that the companies put certain statements or guidance on all informational materials e.g., technical bulletins, grower guides, Internet materials, videos etc.) that may accompany the *B.t.* crop seed at the time of sale, similar to the information that accompanies seeds treated with conventional pesticides. For example, informational labeling materials, such as grower guides, will tell growers that certain resistance management strategies should or must be followed.

Registered *B.t.* Plant-Pesticides

There are currently ten registered products containing *B.t.* endotoxins expressed in potato, corn (field corn, popcorn, and sweet corn), and cotton shown in Table 1.

Registration of *B.t.* Plant-Pesticides: Scientific Data Considerations

For each registered *B.t.* crops, EPA has determined that the proposed use of the plant-pesticide poses no unreasonable adverse effects on the environment. EPA's determination includes includes a thorough review of the human health and environmental risks and a benefit assessment. *B.t.* crops have had safety assessments done by 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. In addition, EPA required the submission of an insect resistance management plan. A summary of the science review findings and regulatory management conclusions for each of the registered *B.t.* plant-pesticides is posted at http://www.epa.gov/biopesticides.

Characterization of the Active Ingredient. Fundamental to EPA's risk assessment of the *B.t.* 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 EPA due to the numerous registered microbial products known to contain the endotoxins in question. 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. 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 FFDCA.

Environmental Fate and Ecological Risk Assessment. Ecological nontarget data needs are driven by exposure to the plant-pesticidal substance expressed in the plant. The pesticidal active ingredient (e.g., the *B.t.* delta-endotoxin and the genetic material necessary for its production) is contained within the plant parts of the crop plant into which it has been genetically engineered. This means that nontarget organisms should have only 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. EPA requires ecological effects data 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 is done on a case-by-case basis.

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 *B.t.* plant-pesticides, 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, effects studies on immature individuals as well as adults may be required if exposure to the *B.t.* 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.

Insect Resistance Management

EPA considers pesticide resistance when making certain regulatory decisions for conventional pesticides under FIFRA. FIFRA provides the Agency the legal authority to manage pesticide resistance. Pesticide resistance and its management have been a factor in many "emergency exemption" (Section 18) decisions that allow for the use of an unregistered pesticide in an emergency situation where significant economic loss would occur under a non-routine situation. Pesticide resistance has also been a factor in the Agency's Special Review Process under Section 6 of FIFRA in determining whether the benefits of maintaining certain pesticide uses outweigh the risks of maintaining these uses. Pesticide resistance management has also been a factor in many Section 3 registration decisions even though there is no formal policy or guidelines on how pesticide resistance management should be considered. One specific example is the specific label statements for resistance management that were developed in consultation with the Pyrethroid Working Group in the early 1980's. More recently, Canada, the U.S., and Mexico joined together under the North American Free Trade Agreement (NAFTA) to develop voluntary pesticide labeling guidelines for pesticide resistance management. The pesticide resistance management guidelines are based on target site mode of action and include a set of of resistance management labeling statements to be modified on a case-by-case basis. Canada has finalized its pesticide resistance management labeling guidelines in October 1999. The U.S. will publish a draft for public comment of very similar guidelines in 2000.

B.t. insect resistance management (IRM) is of great importance because of the threat insect resistance poses to the future use of *B.t.* pesticides. Public interest groups and organic farmers have expressed concern that the widespread planting of these genetically engineered plants will hasten the development of resistance to the pesticidal *B.t.* toxin. In registering transgenic plants expressing *B.t.* endotoxins, EPA has taken extensive and unprecedented measures to significantly reduce the likelihood that insects exposed to *B.t.* endotoxins expressed in transgenic crops will develop resistance. Sound insect resistance management will prolong the life of *B.t.* pesticides, and universal adherence to the plans is to the advantage of growers, producers, and researchers alike. EPA's strategy to address insect resistance is two-fold: (1) mitigate any significant potential for pest resistance development in the field by instituting IRM plans, and (2) better understand the mechanisms behind pest resistance.

As a result of numerous public meetings and consultations with experts in the field, EPA has determined that effective insect resistance management requires a structured refuge/high dose strategy. EPA required that all applicants for registration of transgenic plants expressing *B.t.* endotoxins provide the Agency with IRM plans. Moreover, EPA has mandated certain risk mitigation measures to ensure that selection pressure is effectively managed and the risk of insect resistance development to *B.t.* endotoxins is minimized. The Agency has required or recommended certain research data be developed, development and implementation of structured refuges, annual resistance monitoring, remedial action plans, grower education, and sales and research reporting for certain *B.t.* crops as part of the development and implementation of long-term IRM strategies. EPA's will continue to reevaluate existing IRM strategies prior to the 2001 growing season. Continued development and implementation of long-term IRM strategies will sufficiently mitigate the development of insect resistance to *B.t.* toxins expressed in transgenic crops. EPA's reviews of the IRM strategies for registered *B.t.* plant-pesticides are summarized in the pesticide Fact Sheets available at: http\\:www.epa.gov/pesticides/biopesticides.

Well before registration of the first transgenic plant expressing a *B.t.* endotoxin in 1995, EPA engaged in consultations regarding insect resistance management for transgenic *B.t.* crops at EPA's FIFRA Scientific Advisory Panel (SAP) meetings attended by EPA, USDA, potential registrants, academics, and public interest groups. In addition, potential registrants had been conducting or sponsoring research on the biology and ecology of affected insects and crops (e.g., adult and larval movement, ovipositional and mating behavior, population dynamics, cross-resistance potential, potential resistance mechanisms, refuge strategies, susceptibility etc.) to better understand long-term resistance management of *B.t.* crops to slow or halt the development of insect resistance.

Good resistance management is dependent on multiple tactics to decrease the selection pressure on the target pest(s) and employment of different mortality sources. The 1995 SAP subpanel on plant-pesticides agreed with EPA on the essential elements of an IRM plan: (1) knowledge of pest biology and ecology, (2) appropriate dose expression strategy, (3) appropriate refuges (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 (SAP, 1995). These elements are discussed in more detail in Matten *et al.* (1996) and the EPA White Paper on *B.t.* plant-pesticide resistance management (EPA, 1998).

Subsequent to registration of the first *B.t.* crops in 1995, substantial information has been developed that enhances the Agency's understanding of the requirements of IRM plans. EPA convened threel SAP subpanels (1992,, 1995, 1998), two public hearings in March and May 1997 (EPA, 1998), and two Pesticide Program Dialogue Committee (PPDC) meetings in July 1996 and January 1999 to address, in part, IRM for plants expressing *B.t.* endotoxins. USDA sponsored a *B.t.* crop IRM forum in April 1996. As part of its scientific basis for developing IRM recommendations and requirements for *B.t.* crops, EPA relied on other scientific expert group reports including: the USDA North Central Regional Research Committee NC-205 (NC-205) refuge recommendations (Ostlie *et al.*, 1997; NC-205 Supplement, 1998), a report by the International Life Sciences Institute/Health and Environmental Sciences Institute (ILSI/HESI) (ILSI, 1999), and a publication by the Union of Concerned Scientists (Mellon and Rissler, 1998). EPA and USDA held two public workshops in 1999 on IRM plans for *B.t.* crops and co-authored a position paper on insect resistance management for *B.t.* crops. Academia, growers, industry, and public interest groups discussed the current and future refuge strategies, grower education, compliance concerns, resistance monitoring, and other issues related to IRM for *B.t.* crops. Summaries and proceedings from these two workshops and the EPA/USDA joint position paper are available at http://www.epa.gov/biopesticides.

EPA has also presented its own analysis of *B.t.* plant-pesticide resistance management in a January 1998 paper (EPA, 1998). The White Paper was the focal point of discussion at the February, 1998 SAP subpanel meeting on IRM for *B.t.* crops. The 1998 SAP subpanel recommended that EPA require the use of structured refuges in all registrations of plants expressing *B.t.* endotoxins, unless it can be shown conclusively that such refuges would harm, rather than aid, durability of the resistance management plan (SAP, 1998). The subpanel indicated that acceptable refuge configurations may vary among regions but that a structured refuge should provide sufficient susceptible adult insects to mate with potential *B.t.*-resistant adult insects to dilute the frequency of resistance genes. Similarly, in the referenced reports, symposia, and meetings the consensus that has developed is that a high dose/structured refuge strategy is necessary for mitigating insect resistance to *B.t.* toxins expressed in transgenic crops. The subpanel defined structured refuges to "include all suitable non-B.t. 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 *B.t.* crops are available to the pests or at times when the *B.t.* 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 5×10^{-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.

The 1998 SAP subpanel defined a high dose as 25 times the amount of *B.t.* 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 *B.t.* plants using tissues from non-B.t. 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 $LD_{99.9}$ or higher to assure that 95% of heterozygotes would be killed; (4) Similar to (3) above, but would use controlled infestation with a laboratory strain of the pest that had an LD_{50} value similar to field strains; and (5) Determine if a later larval instar of the targeted pest could be found with an LD_{50} that was about 25-fold higher than that of the neonate larvae. If so, the stage could be tested on the *B.t.* crop plants to determine if 95% or more of the later stage larvae were killed. As discussed below, EPA has taken steps to implement the subpanel's recommendations.

In the past four years, EPA has received no confirmed evidence that field resistance to any *B.t.* endotoxin expressed in these transgenic crops has occurred in any insect species. In 1998, there were approximately 17 million total acres of *B.t.* corn, *B.t.* potato, and *B.t.* cotton planted. Adoption of *B.t.* cotton has been quite high: 1999 - 4.2 million acres, 1998 - 2.5 million acres, 1997 - 2.3 million acres, and 1996 - 1.8 million.

IRM Requirements for Bollgard® Cry1Ac Cotton

The Agency granted the conditional registration of the Cry1Ac delta endotoxins from *Bacillus thuringiensis* subspecies *kurstaki* and the genetic material necessary for its production in cotton to control TBW, CBW, and PBW on October 31,1995. The conditional registration for Cry1Ac will automatically expire at midnight January 1, 2001. EPA required Monsanto to submit annual use and resistance monitoring reports following each growing season. EPA also required the following research data: target pest biology and ecology data, non-cotton hosts as refuge, cross-resistance potential, influence of *B.t.* cotton on secondary lepidopteran pests, Cry1Ac expression information in different plant parts as it relates to the target lepidopteran pests. EPA also required Monsanto to continue development and distribution of grower education materials.

As a condition of the 1995 registration, EPA required two structured refuge options for *B.t.* cotton to mitigate the development of tobacco budworm (TBW, *Heliothis virescens (Fabricius)*), cotton bollworm (CBW, *Helicoverpa zea (Boddie)*), and pink bollworm (PBW, *Pectinophora gossypiella (Dyar)*) resistance. To decrease the potential for target insect pests to become resistant to Cry1Ac, two specific refuge options were mandated by EPA as requirements of registration to mitigate the development of resistance. "Option A: For every 100 acres of cotton with the Bollgard gene planted, plant 25 acres of cotton without the Bollgard that can be treated with insecticides (other than foliar B.t.k products) that control the tobacco budworm, cotton bollworm, and pink bollworm. Option B: for every 100 acres of cotton with the Bollgard gene planted, 4 acres of cotton without the Bollgard gene that cannot be treated with acephate, amitraz, endosulfan, methomyl, profenofos, sulprofos, synthetic pyrethroids, and/or *Bacillus thuringiensis* subsp. *kurstaki (B.t.k)* insecticides labeled for the control of tobacco budworm, cotton bollworm, and pink bollworm. The refuge acreage must be managed similarly to Bollgard cotton." The intent of Option B was that there would be no spraying of the prohibited pesticides for TBW, CBW, or PBW, on the 4% untreated external refuge.

In addition, if cotton with the Bollgard gene exceeds 75% of the total amount of the cotton planted in any single county or Parish in any year, growers in that county or Parish choosing to use the 4% untreated refuge option the following year will be required to plant the 4% refuge within one mile of the respective Bollgard cotton field. Similarly, if EPA grants a registration for cotton containing the *B.t.k.* insect control protein to another company, EPA will determine whether the combined acreage of cotton containing the B.t.k insect control protein exceeds 75% of the total amount of the cotton planted in a single county or Parish and inform the registrants that the 4% refuge must be planted in a single county or Parish and inform the registrants that the 4% refuge must be planted within one mile of the respective Bollgard cotton or other *B.t.k* cotton fields. Monsanto requires that growers sign a grower contract. Growers must plant at least one of these two structured refuges on their farm according to the terms of the grower contract. Monsanto has notified growers in an affected county or parish whether the 75% trigger has been exceeded and inform growers that if they choose the 4% unsprayed refuge that it must be planted within one mile of the *B.t.* cotton fields.

EPA also requires annual resistance monitoring for TBW, CBW, and PBW. The resistance monitoring program measures changes in susceptibility to the Cry1Ac toxin relative to the baseline susceptibility data collected in different geographic areas. 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. Remedial action plans to address confirmed (or suspected) insect resistance are required by the Agency. Monsanto is required to instruct customers to contact the company regarding unexpected levels of TBW, CBW, or PBW damage or if resistance is suspected. Monsanto is to investigate and identify the cause of such damage. Based on these investigations, appropriate remedial action is required to mitigate resistance. Resistance monitoring will be intensified in instances of suspected or confirmed resistance. Any confirmed incidents of resistance are required to be reported to the EPA under the terms and conditions of the registration and also under FIFRA 6(a)2. Monsanto has instructed its customers to have regular surveillance programs and report any unexpected levels of TBW, CBW, and PBW damage to them and to their local extension agents. Remedial actions include: inform customers and extension agents in the affected areas of resistance problems, implementing alternative means to reduce or control the resistant populations, increasing monitoring in the affected areas, modifying refuges in the affected areas, and ceasing of sales in the affected and bordering counties. Industry cooperation with extension and academics entomologists and consultants is considered important in communicating specific information of definitions of "unexpected damage" and appropriate remedial action.

Monsanto provided the Agency results of the TBW, CBW, and PBW resistance monitoring programs for the 1996, 1997, and 1998 growing seasons. Resistance monitoring results for the 1999 growing season are due January 2000. Monsanto also reported to EPA suspected incidents of bollworm resistance to *B.t.* cotton in July 1996. Upon further investigation, available scientific information indicated that TBW and CBW susceptibilities to the Cry1Ac were unchanged in the affected locations after the 1996 cotton growing season. The Cry1Ac expression in Bollgard cotton was as expected. There was no evidence for TBW or CBW resistance to the Cry1Ac toxin. Thus, the reports of suspected resistance were unconfirmed. Results indicate no evidence of TBW, CBW, or PBW resistance to the Cry1Ac toxin in the field (EPA, 1998; Sumerford *et al.,* 1999; Simmons *et al.*, 1998; Patin *et al.*, 1999). However, Sumerford *et al.* (1999) report that there is evidence that CBW populations in South Alabama, Mississippi Delta, Georgia, Florida Panhandle, and South Carolina may have developed increased tolerance (about 10-fold) to the Cry1Ac toxin during the three-year period from 1996 to 1998. But, increased tolerance should not be interpreted as resistance. There is no evidence of field failure due to either TBW or CBW resistance. However, these results do indicate that factors selecting for CBW resistance may already be increasing in the field and further analysis is necessary. Further investigation is planned for the 2000 growing season.

<u>Reevaluation of Existing IRM Plans for 2001</u>

The content and implementation of IRM plans continues to be a focus for EPA, and our stakeholders. Because of our continuing concerns about the potential development of insect resistance to *B.t.* , and in order to ensure that *B.t.* products remain effective for all farmers, a refuge must be established on a per farm basis. The issue of refuge size and deployment continues to be investigated extensively by a number of groups from industry, academia, public interest groups, and the federal government. We are working with USDA, academia, growers, the registrants, and public interest groups to ensure that new genetically modified pesticide products can be used without the development of pest resistance.

Several new IRM strategies have been proposed for *B.t.* cotton. All of these strategies and others submitted to the Agency will be evaluated in 2000.

> *For TBW:* The Research and Extension Entomologists from Texas/Oklahoma to East Coast (Hardee *et al.,* 1999) state in their draft document entitled "*B.t.* Cotton for Management of Tobacco Budworm and Bollworm: Continued Effectiveness by Management Resistance" that "computer simulation models, along with limited evidence from field and laboratory studies, have predicted that the 4% unsprayed refuge or the 20% sprayed refuge options recommended on the original *B.t.* cotton label (Bollgard® cotton) will not adequately delay resistance in bollworm, and perhaps tobacco budworm." This group recommends three refuge options: 1) "2:1 Sprayed Refuge with Site Restrictions, 2) "In-Field 90/10 Refuge with Spray Application Restrictions", and 3) "Producers with Numerous Small Fields <25 Acres Option: 90 *B.t.* :10 Embedded Refuge within a Square Mile Area." These research and extension experts state that "there are many options for less restrictive IRM plans, such as a 20% sprayed refuge or a 5% in-field refuge. These options may better suit business interests, short term economics, on-farm logistics, and farmers in high tobacco budworm/bollworm areas, especially where pyrethroid resistance is prevalent."
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> Gould and Tabashnik (1998) proposed two refuge options: 1) "IPM option with 50 percent non-*B.t.* cotton. For every acre of cotton with the Bollgard gene (i.e., *B.t.* cotton) planted, plant at least one acre of cotton without the Bollgard gene (i.e., non-*B.t.* cotton). To maintain an appropriate spatial scale, at least 50 percent of the cotton within each one-mile-by-one-mile-square area planted by each grower must be *B.t.* cotton. This non-*B.t.* cotton can be treated with insecticides other than foliar *B.t.* products, including insecticides that control tobacco budworm and cotton bollworm. This insecticide use must be based on scouting conducted as part of an IPM program." 2) "A 16.7 percent unsprayed-refuge option. Within every 48 rows of cotton planted, plant at least eight rows of non-*B.t.* cotton. The non-*B.t.* cotton must planted in sets of two or more adjacent

row." The non-*B.t.* cotton refuge cannot be treated with insecticides unless the entire field is treated in the same manner.

For PBW: The Arizona *B.t.* Cotton Working Group for PBW Resistance Management recommended two options: 80:20 External Sprayed Refuge (deployment within one quarter section - 160 acres) and 90:10 In-field refuge (at least one row out of six rows must be non-*B.t.* cotton).

Gould and Tabashnik (1998) recommended two options for PBW resistance management: "1) IPM options with 50 percent non-*B.t.* cotton: For every acre of cotton with the Bollgard gene planted, plant at least one acre of non-*B.t.* cotton in that field. 2) A 16.7 percent unsprayed-refuge option: within every 48 rows of cotton planted, plant at least eight row of non-*B.t.* cotton. The non-*B.t.* cotton must planted in sets of two or more adjacent rows."

The California Cotton Pest Control Board recommended that 100% *B.t.* cotton be allowed for PBW suppression programs in San Joaquin Valley, Imperial Valley, and Palo Verde Valley.

EPA believes that scientifically-sound long-term resistance management strategies are essential to the survival of *B.t.* plant-pesticides, protection of *B.t.* microbial pesticides, and reduction in the risks from the use of pesticides. Work is underway to reevaluate all of the existing IRM plans for *B.t.* crops (*B.t.* corn and *B.t.* cotton) prior to their 2001 expiration dates. The Cry1Ac cotton registration expires January 1, 2001. EPA will also consider the impact stacked gene products (e.g., products with more than one *B.t.* gene for control of certain lepidopteran pests) may have on insect resistance management strategies. EPA will have a public process in 2000 to reevaluate the current IRM plans prior to making regulatory decisions for the 2001 growing season. This process will include a public comment on EPA's review of IRM plans and requirements and a Science Advisory Panel meeting to peer review the scientific merits of EPA's analysis. EPA will continue to work with stakeholders from industry, academia, trade organizations, public interest groups, and government agencies to address long-term insect resistance management for *B.t.* crops.

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

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Table 1. Registered B.t. Plant-Pesticides for Full Commercial Use

Events/ Products	**Date Registered**	**Toxin**	**Crop**	**Company**
NewLeaf®	May 1995	Cry3A	Potato	Monsanto
NewLeaf Plus®	Nov 1998	Cry3A and potato leaf roll virus replicase	Potato	Monsanto
176	Aug 1995 Mar 1998	Cry1Ab	Field Corn Popcorn	Novartis
176	Aug 1995	Cry1Ab	Field Corn	Mycogen/ Dow
BT11	Aug 1996	Cry1Ab	Field Corn	Novartis
BT11	Mar 1998	Cry1Ab	Sweet Corn	Novartis
MON810	Dec 1996	Cry1Ab	Field Corn	Monsanto
DBT-418	Mar 1997	Cry 1Ac	Field Corn	DeKalb
CBH-351	May 1998	Cry9C	Field Corn	AgrEvo
Bollgard®	Oct 1995	Cry1Ac	Cotton	Monsanto