BIOPESTICIDES REGISTRATION ACTION DOCUMENT

(*BACILLUS THURINGIENSIS* VAR. *AIZAWAI* CRY1F AND THE GENETIC MATERIAL (FROM THE INSERT OF PLASMID PGMA281) NECESSARY FOR ITS PRODUCTION IN COTTON AND *BACILLUS THURINGIENSIS* VAR. *KURSTAKI* CRY1AC AND THE GENETIC MATERIAL (FROM THE INSERT OF PLASMID PMYC3006) NECESSARY FOR ITS PRODUCTION IN COTTON)
(Chemical PC Codes 006512 and 006513, respectively)

September 2005

U.S. Environmental Protection Agency
Office of Pesticide Programs
Biopesticides and Pollution Prevention Division

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BACILLUS THURINGIENSIS CRY1AC/CRY1F COTTON (WIDESTRIKE) REGISTRATION ACTION TEAM

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I. Overview

A. Executive Summary

EPA has conditionally registered Dow AgroSciences' new active ingredients, *Bacillus thuringiensis* var. *aizawai* Cry1F and the genetic material (from the insert of plasmid pGMA281) necessary for its production in cotton and *Bacillus thuringiensis* var. *kurstaki* Cry1Ac and the genetic material (from the insert of plasmid pMYC3006) necessary for its production in cotton. The Agency has determined that the use of these pesticides is in the public interest and that they will not cause any unreasonable adverse effects on the environment during the time of this conditional registration.

Cotton is the most important fiber crop in the U.S. and lepidopteran insects are the main insect problem for that crop. According to USDA/NASS data, 68% of all acres planted to cotton in the U.S. were treated with insecticides, accounting for over 21 million pounds of applied pesticide chemicals. Assessing the potential benefits from adoption of WideStrike® cotton, EPA compared the efficacy of WideStrike® cotton to both other chemical controls and other Bt cotton products, evaluated the human health and environmental benefits compared to these registered alternatives, estimated the grower benefits, insect resistance management benefits, and estimated the chemical pesticide use reduction from adoption of this product. EPA made a determination that the registration of the *Bacillus thuringiensis* var. *aizawai* Cry1F and the genetic material (from the insert of plasmid pGMA281) necessary for its production in cotton and *Bacillus thuringiensis* var. *kurstaki* Cry1Ac and the genetic material (from the insert of plasmid pMYC3006) necessary for its production in cotton (WideStrike® cotton) was in the public interest and that the benefits outweigh the risks.

Human health and ecological safety, insect resistance management, public interest finding, and benefits assessments conducted in support of this registration are summarized in this document. Data submitted under the EUPs were also relied upon as part of the Agency's review for the full section 3 commercial registration. Prior to registration of this new two toxin product, WideStrike® cotton, a Science Advisory Panel (SAP) was convened to discuss the Agency's review of the product characterization, ecological toxicity, and insect resistance management data submitted by the company. As detailed later in this document, the Agency has considered those recommendations from the SAP Report, dated August 19, 2004, that are directly responsive to the specific questions that the Agency presented to the SAP. Any additional information and recommendations provided by the SAP that were not directly responsive to the specific questions presented by the Agency are nonetheless viewed as valuable and will be considered, as appropriate, in connection with future actions.

As noted above, WideStrike® cotton is comprised of the *Bt* proteins Cry1Ac and Cry1F and is intended to be more efficacious in the protection of cotton from feeding by tobacco budworm (*Heliothis virescens*, TBW), pink bollworm (*Pectinophora gossypiella*, PBW), cotton bollworm (*Helicoverpa zea,* CBW), cabbage looper (*Trichoplusia ni,* CL), soybean looper (*Pseudoplusia*

includens, SL), beet armyworm (*Spodoptera exigua,* BAW), fall armyworm (*Spodoptera frugiperda,* FAW), yellowstriped armyworm (*Spodoptera ornithogolli*, YAW). Six additional minor lepidopteran cotton pests are also controlled: black cutworm (*Agrotis ipsilon*, BCW), citrus peelminor (*Marmara gulosa*, CPM), ominorous leafroller (*Platynota stultana*, OLR), saltmarsh caterpillar (*Estigmene acrea,* SC), cotton leaf perforator (*Bucculatrix thurbeiella,* CLP), and European corn borer (*Ostrinia nubilalis,* ECB*).*

This conditional registration will automatically expire on September 30, 2009.

B. Use Profile

- Active Ingredients: *Bacillus thuringiensis* var. *aizawai* Cry1F and the genetic material (from the insert of plasmid pGMA281) necessary for its production in cotton and *Bacillus thuringiensis* var. *kurstaki* Cry1Ac and the genetic material (from the insert of plasmid pMYC3006) necessary for its production in cotton

- Trade and Other Names: Mycogen Brand Cry1F (synpro)/Cry1Ac (synpro) Construct 281/3006 Cotton; WideStrike® cotton

- OPP Chemical Codes: 006512 and 006513

- Basic Manufacturer: Dow AgroSciences LLC
  9330 Zionsville Road
  Indianapolis, IN 46268

- Type of Pesticide: Plant-incorporated protectant

- Uses: Cotton

- Target Pest(s): Cotton bollworm, tobacco budworm, pink bollworm, soybean looper, cabbage looper, beet armyworm, fall armyworm, southern armyworm, black cutworm, citrus peelminer, cotton leafperforator, European corn borer, ominivorous leafroller, and saltmarsh caterpillar.

C. Regulatory History

Dow AgroSciences submitted an application for a full commercial use under Section 3 of the Federal Insecticide, Fungicide, and Rodenticide Act, as amended (FIFRA) for WideStrike® cotton [Mycogen Brand Cry1F (synpro)/Cry1Ac (synpro) Construct 281/3006 Cotton] on November 13, 2002. WideStrike®[1] expresses both the Cry1F and Cry1Ac synthetic insecticidal crystalline proteins (ICPs). Previously, Dow AgroSciences submitted an application on November 5, 2001 for an Experimental Use Permit (EUP) under Section 5 of the Federal Insecticide, Fungicide, and Rodenticide Act, as amended, (FIFRA) for WideStrike® cotton to be planted on 407.2 acres. This EUP was subsequently amended to increase the acreage to 2826 acres on November 6, 2002. The EUP was granted on April 11, 2003.

An existing tolerance exemption, CFR 40 Section 180.l155, exists for *Bacillus thuringiensis* subsp. *kurstaki* Cry1Ac and the genetic material necessary for its production in all plants. An existing tolerance exemption, CFR 40 Section 180.1151, exists for phosphinothricin acetyltransferase (PAT) and the genetic material necessary for its production in all plants. The PAT in Mycogen Brand Cry1F (synpro)/Cry1Ac (synpro) Construct 281/3006 Cotton is covered by the existing tolerance exemption, CFR 40 Section 180.1151. Similarly, data provided by Dow AgroSciences are adequate to support the existing tolerance exemption for Cry1Ac.

In the Federal Register of August 11, 2004 (69 FR 48870; FRL-2), EPA issued a notice pursuant to section 408(c)(3) of the FFDC, U.S.C. 346a(d)(3), announcing the filing of a pesticide tolerance petition (PP 3F6785) by Dow AgroSciences. The petition requested that 40 CFR 174 be amended by establishing a tolerance exemption from the requirement of a tolerance for residues of *Bacillus thuringiensis* subsp *aizawai* strain PS811 (Cry1F insecticidal control protein) and the genetic material necessary for its production in cotton when used as a plant-incorporated protectant. This notice included a summary of the petition prepared by the petitioner Dow AgroSciences.
The only comments received in response to the notice of filing were from the National Cotton Council supporting this petition. A tolerance exemption was issued on September 30, 2004 (69 FR 58280 under 40 CFR 174.455 for the *Bacillus thuringiensis* Cry1F protein and the genetic material necessary for its production in or on cotton. Originally, a temporary tolerance exemption for the Cry1F protein (and the genetic material necessary for its production in or on cotton) was issued by the Agency on April 30, 2003 (40 CFR 180.1227). This temporary tolerance exemption, 40 CFR 180.1227, was extended by the Agency on March 31, 2004 (68 FR 16819) to May 1, 2005. The temporary tolerance exemption was replaced by the permanent tolerance exemption for the *Bacillus thuringiensis* Cry1F protein and the genetic material necessary for its production in or on cotton on September 30, 2004 (40 CFR 174.455).

An existing tolerance exemption, CFR 40 Section 180.l155, exists for *Bacillus thuringiensis* subsp. *kurstaki* Cry1Ac and the genetic material necessary for its production in all plants. An existing tolerance exemption, CFR 40 Section 180.1151, exists for phosphinothricin

[1]WideStrike® is a trademark of Dow AgroSciences.

acetyltransferase (PAT) and the genetic material necessary for its production in all plants. The PAT in Mycogen Brand Cry1F (synpro)/Cry1Ac (synpro) Construct 281/3006 Cotton is covered by the existing tolerance exemption, CFR 40 Section 180.1151. Similarly, data provided by Dow AgroSciences are adequate to support the existing tolerance exemption for Cry1Ac.

II. Science Assessment

A. Product Characterization

Dow AgroSciences transformed Acala cotton line GC510 with plasmids pAGM281 and pMYC3006. Cotton event 281-24-236 (Cry1F) resulted from an insertion from pAGM281 of one intact copy of *cry1F* and one intact copy of the *pat* gene (plant selectable marker gene, phosphinothricin acetyltransferase, PAT). Cotton event 3006-210-23 (Cry1Ac) resulted from an insertion from pMYC3006 of one intact copy *of cry1Ac* and one intact copy of the *pat* gene. These two Acala cotton lines, Event 281-24-236 (Cry1F) and Event 3006-210-23 (Cry1Ac) were separately backcrossed three times with cotton line PSC355 followed by one generation of self-pollination to yield the BC3F1generation. The two BC3F1 events were then intercrossed and self-pollinated to the F3 generation, forming cottonseed designated 281-24-236/3006-210-23, which contains the genes for expression of Cry1F, Cry1Ac, and PAT proteins designated as WideStrike® (MXB-13). WideStrike® cotton is intended to protect cotton from feeding by three key lepidopteran pests of cotton in their respective geographies: TBW, PBW, and CBW. In addition, several other lepidopteran pests are controlled by this product: CL, SL, BAW, FAW and SAW.

The characterization data submitted by the registrant provides adequate product information to guide the risk assessment. These data indicate that plant-produced and bacterially-produced Cry1F, Cry1Ac, and PAT proteins are biologically, biochemically, and immunologically equivalent. Southern blot data of restriction enzyme digests suggest that the Cry1Ac event, Cry1F event, and the pyramided Cry1F/Cry1Ac cotton event all contain a single, unique, insertion of the transgenic DNA from the appropriate plasmids. An additional hybridizing fragment of *pat* gene was integrated into the cotton genome from pAGM281.

The field expression data for Cry1F (synpro), Cry1Ac (synpro) and phosphinothricin acetyltransferase (PAT) proteins in transgenic cotton plants, cottonseed and cottonseed processed products provides quantitative data on the expression of Cry1F and Cry1Ac proteins in different cotton plant tissues which are: young leaves, squares,, flowers, boll, whole plant, pollen, nectar, root, seed, and cottonseed process fractions consisting of kernel, hulls, meal and oil. These data also contain data from the compositional analysis of leaf, square, seed, and cottonseed process fractions consisting of kernel, hulls, meal and oil. The soluble, extractable Cry1F, Cry1Ac and PAT proteins were measured using ELISA methods with a limit of quantitation ranging from 0.001-0.4 ng protein/mg sample weight. Expression levels were measured in several different transgenic cotton lines: Cry1F alone (Event 281-24-236), Cry1Ac alone (Event 3006-210-23), and Cry 1F/Cry1Ac pyramided (Event 281-24-236/3006-210-23), as well as the herbicide

resistant selectable marker that expresses the PAT protein. The Cry1Ac and Cry1F proteins were detected in all matrices except nectar, meal and oil, Cry1Ac was also not detected in hulls. Mean Cry1Ac expression was approximately three- to twenty-times lower than Cry1F expression in leaves, squares, flowers, whole plant, boll, and seed tissue, depending on the tissue. Pollen was the only tissue in which Cry1Ac expression was higher than Cry1F expression. Expression levels of individual Cry1F and Cry1Ac proteins were similar for the single event and two-protein cotton lines. PAT proteins were detected in the cotton samples from the Cry1F event and the Cry1F/Cry1Ac event, but generally not detected in the Cry1Ac event samples.

Highest Cry1Ac mean expression was observed in young leaves and squares, 1.82 ng Cry1Ac/mg tissues, and in flowers, 1.83 ng Cry1Ac/mg tissue. Mean Cry1Ac expression was 1.31 ng Cry1Ac/mg tissue in terminal leaves, and 0.55 ng Cry1Ac/mg tissue in seeds. Mean Cry1Ac expression in root tissue ranged from N.D. to 0.2 ng Cry1Ac/mg tissue. Mean Cry1Ac expression in pollen was 1.45 ng Cry1Ac/ mg pollen.

Highest Cry1F mean expression was observed in young leaves, 6.81 ng Cry1F/mg tissue, and terminal leaves, 8.19 ng Cry1F/mg tissue. Mean Cry1F expression was 4.88 ng Cry1F/mg tissue in squares, 5.44 ng Cry1F/mg tissue in flowers, 3.52 ng Cry1F/mg tissue in bolls, and 4.13 ng Cry1F/mg tissue in seeds. Mean Cry1F expression in root tissue was 0.5 to 0.9 ng Cry1F/mg tissue. Mean Cry1F expression in pollen was less than the limit of quantitation, <0.15 ng Cry1F/ mg pollen.

B. HUMAN HEALTH ASSESSMENT

The health effects assessment concludes that there is a reasonable certainty that no harm will result from exposure to Cry1F and Cry1Ac. For purposes of the dietary risk assessment, the maximum levels of expression in cottonseed (cotton processed fraction) were 0.46 and 3.1 ng protein/mg tissue fresh weight for Cry1Ac and Cry1F proteins in the Cry1F/Cry1Ac cotton lines, respectively, based on the expression data (see above).

1. Mammalian Toxicity and Allergenicity

Based upon the human health data provided, there does not appear to be a significant risk of toxic effects and/or allergenic effects to humans or animals due to exposure to the Cry1F (synpro) and Cry1Ac (synpro) proteins. Based on a review of the data, there is a reasonable certainty of no harm to humans and animals posed by these proteins in connection with their proposed uses in this product. Mammalian toxicology data are available to examine the potential effects of Cry1F and Cry1Ac proteins on human health. *Bt* microbial pesticides, containing Cry proteins other than Cry1F and Cry1Ac, have been applied for more than 30 years to food and feed crops consumed by the U.S. population. These data would also support other Cry1F and Cry1Ac plant-incorporated protectant human health assessments. Adequate information was submitted to show that the Cry1F and Cry1Ac test material derived from microbial cultures were biochemically and functionally similar to the proteins produced as the plant-incorporated protectant ingredients.

The Cry1F and Cry1Ac proteins are classified as Toxicity Category III: LD_{50}> 700 mg/kg body weight for Cry1Ac, LD_{50}> 600 mg/kg body weight for Cry1F and LD_{50}> 375 mg Cry1F/kg body weight and LD_{50}>350 Cry1Ac mg/kg body weight for the pyramided Cry1F/Cry1Ac proteins. The Cry1Ac and Cry1F proteins are not stable to digestion in simulated gastric fluid (<1 min), nor do they share any significant sequence similarity to known toxins or allergens using an eight amino acid step-wise comparison. In addition, Cry1F and Cry1Ac proteins have not been implicated in toxic and/or allergenic reactions in humans or animals.

2. Dietary Exposure and Risk

Humans may consume cotton products as a food (cottonseed oil) or as food ingredients (cottonseed fatty acids, cooked and partially defatted cottonseed flour, and decorticated ground cottonseed kernels); however, human consumption of the cotton products is limited. Consumption data from the USDA Continuing Survey of Food Intake by Individuals (CSFII) 1994-98, as used within DEEM (Dietary Exposure Estimation Model version 7.73, Novigen Sciences, Inc., Washington, DC, contains information on daily dietary consumption of cottonseed oil (food code 290) and cottonseed meal (food code 291). These data indicate mean dietary consumption of cotton products in the US diet is 2 g/day (95^{th} percentile, 5 g/day). Cottonseed oil is the exclusive contributor to diet at the higher percentiles of consumption.

In a worst case scenario, the estimate of human dietary exposure conservatively assumes dietary consumption of cotton products where 100% of diet contains Cry1Ac and Cry1F protein at the maximum levels expressed in cottonseed (whereas, the dominant food consumed is cottonseed oil where actual protein content is nil). The worst case upper bound on combined Cry1Ac and Cry1F protein intake in the human diet is <10 ng/g (ppb) of daily consumption. The exposure estimate does not account for the rapid digestibility of Cry1Ac and Cry1F protein (non-detectable in <1 min). Assuming the average estimated lifetime dietary intake of 2141 g food/day for the US population (USDA, 1998), the combined daily intake of Cry1Ac and Cry1F protein from consumption of cotton products is < 10 ppb (1.1 and 7.2 ng/g diet, respectively).2 Analysis of cottonseed processed products indicates that there is no detectable Cry1Ac and Cry1F protein in either toasted meal or refined oil.

3. Animal Dietary Exposure and Risk

Animals may consume cottonseed, cottonseed meal, cottonseed hulls, or cotton gin by-products as a portion of their diet. Animal feeding of whole cottonseed (22% protein) is constrained by the level of gossypol and polyunsaturated oil content. Cottonseed is not fed to poultry. Due to gossypol toxicity, swine are only fed cottonseed on an infrequent basis. Cotton gin by-products contain from 4 to 8% protein. The actual use of cotton gin by-products in U.S. beef feed is about 5% of the diet.

The worst case exposure from consumption of Cry1Ac and Cry1F residues in cotton products is that of dairy cattle consuming 100% of their total protein as cottonseed meal (CSM). This, of course, is an unrealistic scenario based on the previously mentioned gossypol toxicity limitations for cottonseed. Subsequent analysis of cottonseed processed products indicates that there is no detectable (i.e., < 0.1 ng/mg) Cry1Ac and Cry1F protein in toasted meal. Based on the toxicity and the exposure analyses, the animal dietary risk from consumption of Cry1Ac and Cry1F residues in cottonseed-based feeds is negligible.

4. Exemption from the Requirement of a Tolerance for the Cry1F, Cry1Ac, and PAT Proteins

An existing tolerance exemption, 40 CFR Section 180.l155, exists for *Bacillus thuringiensis* subsp. *kurstaki* Cry1Ac and the genetic material necessary for its production in all plants. Similarly, an existing tolerance exemption, 40 CFR Section 180.1151, exists for the PAT protein and the genetic material necessary for its production in all plants. The PAT in Mycogen Brand Cry1F (synpro)/Cry1Ac (synpro) Construct 281/3006 Cotton is covered by the existing tolerance exemption, 40 CFR Section 180.1151. Data provided by Dow AgroSciences support issuance of a tolerance exemption for the *Bacillus thuringiensis aizawai* Cry1F protein and the genetic material necessary for its production in or on cotton. The analytical methods for the Cry1Ac and

2 Human exposure example calculation for Cry1Ac (dietary intake basis):

0.46 ng Cry1Ac/mg CP X 5g CP consumed/day X 1000 mg/g X day/2141g diet = 1.1 ng Cry1Ac consumed/g diet

Cry1F proteins are acceptable.

5. Summary of Product Characterization and Mammalian Toxicity/Allergenicity Data

A summary of the product characterization and human health safety data is presented in **Table 1.**

Table 1. Summary of Product Characterization and Human Health Safety Data

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Product Characterization and Identity (885.1100)		Results
458084-08	Field Expression of Cry1F (synpro), Cry1Ac (synpro), and Phosphinothricin Acetyltransferase (PAT) Proteins in Transgenic Cotton Plants, Cottonseed, and Cottonseed Processed Products; and Compositional Analysis of Cottonseed and Cottonseed Processed Products	Acceptable
458084-01	Molecular Characterization of Cry1F (synpro) Transgenic Cotton Event 281-24-236	Acceptable
458084-02 and 459227-01	Molecular Characterization of Cry1Ac (synpro) Transgenic Cotton Event 3006-210-23	Acceptable
458084-03	Molecular Characterization of Cry1F (synpro)/Cry1Ac (synpro) Pyramided Transgenic Cotton Event 281-24-236/3006-210-23	Acceptable
458186-03	Expression of the Partial PAT Open Reading Frame in B.t. Cry1F Cotton Event 281-24-236	Acceptable
458084-04	Purification and Characterization of Cry1Ac Delta-Endotoxin from Transgenic Cotton Event 3006-210-23	Acceptable
458084-05	Characterization of Phosphinothricin Acetyltransferase (PAT) from Recombinant *Escherichia coli* and Transgenic Cotton	Acceptable

Product Characterization and Identity (885.1100) cont...		Results
458084-06	Biological Equivalency of Event 3006-210-23 Cotton- and *Pseudomonas*-Expressed Cry1Ac B.t. Insecticidal Crystal Protein	Acceptable
456079-02	Product Characterization of Cry1F (synpro) Protein	Acceptable
456079-01	Product Characterization of Cry1Ac (synpro) Protein	Acceptable
455423-03	Biological Equivalency of Transgenic Cotton- and *Pseudomonas*-expressed Cry1F Proteins	Acceptable
455423-04	Biological Equivalency of Transgenic Cotton- and *Pseudomonas*-expressed Cry1Ac Proteins	Acceptable
455423-05	Biochemical Characterization of Cry1F derived from Transgenic Cotton and *Pseudomonas fluorescens*	Acceptable
455423-07	Cotton-Insect-Pest Susceptibility Study - Microbial B.t. Cry1F (synpro)	Acceptable

	Protein	
455423-08	Cotton-Insect-Pest Susceptibility Study - Microbial B.t. Cry1Ac (synpro) Protein	Supplemental, not upgradeable
Human Toxicity (885.3050) and Allergenicity		
455423-12	Acute Oral Toxicity: Cry1F (synpro)Bacterial Protein	Acceptable
455423-13	Acute Oral Toxicity: Cry1Ac (synpro) Bacterial Protein	Acceptable
455423-14	Acute Oral Toxicity: Cry1F (synpro) + Cry1Ac (synpro) Bacterial Proteins	Acceptable
455423-09	Comparison of Amino Acid Sequence with Known Allergens: Cry1F (synpro) Protein as Expressed in Cotton	Acceptable
455423-10	Comparison of Amino Acid Sequence with Known Allergens: Cry1Ac (synpro) Protein as Expressed in Cotton	Acceptable
455423-11	Comparison of Amino Acid Sequence with Known Allergens: PAT Protein as Expressed in Cotton	Acceptable
455423-18	*In vitro* Digestibility of Bacterially-derived Cry1F (synpro)	Acceptable
455423-19	*In vitro* Digestibility of Bacterially-derived Cry1Ac (synpro)	Acceptable
455423-20	Thermolability of Cry1F (synpro) Delta-Endotoxin	Acceptable
455423-21	Thermolability of Cry1Ac (synpro) Delta-Endotoxin	Acceptable
Human Toxicity (885.3050) and Allergenicity (cont...)		Results
458084-16	*In Vitro* Simulated Gastric Fluid Disgestibility Study of Recominant Phosphinothricin Acetyltransferase (PAT)	Acceptable
Analytical Methods		
458084-24	Development and Characterization of Enzyme-Linked Immunosorbent Assay (ELISA) for the Detection of Cry1Ac Protein	Acceptable
458084-22	Independent Laboratory Validation of Method GRM 02.11, "Determination of Cry1Ac Insecticidal Crystal Protein in Cotton Tissues by Enzyme-Linked Immunosorbent Assay"	Acceptable
456759-01	Lateral flow test kit method validation for the detection of the Cry1F (synpro) protein in cotton seed	Acceptable
458084-23	Development and characterization of enzyme linked-immunosorbent assay (ELISA) for the detection of Cry1F protein	Acceptable
456759-02	Lateral flow test kit method validation for the detection of the Cry1Ac (synpro) protein in cotton seed	Acceptable

C. ENVIRONMENTAL ASSESSMENT

The Agency has conducted an environmental hazard assessment of the WideStrike® (MXB-13) transgenic cotton line containing two PIPs (Cry1F/Cry1Ac). The assessment includes effects on wildlife, gene flow to related wild plants, development of weediness, fate of Cry1F/Cry1Ac proteins in the environment and effects on endangered species. The assessment is based on data submitted to the Agency during the developmental stages of the transgenic cotton lines, additional data submitted for registration, FIFRA Scientific Advisory Panel (SAP) recommendations, consultations with scientific experts, and public comments received.

Based on the evaluation of the submitted limit dose testing data and information on the general biology of *Bt* Cry proteins, no unreasonable adverse effects on the flora and fauna of the cotton agroecosystems are expected from the cultivation of MXB-13 transgenic cotton. Specific data are cited relating to aquatic and terrestrial wildlife, Cry protein fate in soils, potential effects on soil biota and field census data examining the effects on non-target foliar insects, and endangered or threatened species hazard assessment, particularly Lepidoptera listed by the United States Fish and Wildlife Service (USFWS). The submitted studies examined the effects of the Cry1F and Cry1Ac proteins separately and in combination to detect any possible synergistic effects. No synergistic effects or increase in non-target host range as a result of pyramiding were seen.

Summaries of these studies are presented here in both tabular **(Table 2)** and more detailed descriptive format. EPA concluded, based on the June 2004 SAP that extended field data were not needed from a regulatory perspective.

1. Non Target Wildlife Hazard Assessment

a) The Hazard Assessment

The Agency assesses the toxicity of a Cry protein (*B.t.* endotoxin) to representatives of potentially exposed non-target organisms by a tiered testing system starting with Tier I single species high dose laboratory data using mortality as the end point. Negative results from tests using this approach provide a high degree of confidence that no unreasonable adverse effects are likely to occur.

The maximum hazard dose approach is based on a safety factor times the maximum amount of active ingredient expected to be available to terrestrial and aquatic plants and animals in the environment (the expected environmental concentration, or EEC). Therefore, data that establishes an LC_{50}, ED_{50}, or LD_{50} that is greater than the maximum hazard dosage level (e.g. LD_{50} >10 X EEC) is sufficient to evaluate adverse effects and lower dose testing is not necessary.

Bt Cry endotoxins are proteins that do not have the potential to bioaccumulate and thereby result in delayed adverse effects. An accumulation through the food chain is therefore not expected to take place, and there are no data to support this possibility for protein substances. The basic

biological properties of proteins also make *Bt* Cry proteins readily susceptible to metabolic, microbial, and abiotic degradation once they are ingested or excreted into the environment. Although there are reports of Cry protein binding by certain soils under certain circumstances, the bound Cry proteins are also reported to be rapidly degraded by microbes upon elution. The same sources also report that *Bt* proteins in the soil in *Bt* crop fields have no detectable effect on soil invertebrates or culturable microbial flora. In addition, *Bt* Cry proteins do not have any characteristics in common with persistent, bioaccumulative chemicals that are transferred through the food chain. Therefore, chronic effects testing of protein substances are not routinely performed.

b) Hazard Assessment of Cotton Expressing Cry1F and Cry1Ac Insecticidal Crystalline Proteins to Non-target, Beneficial and Endangered Wildlife

1. Summary of Non-Target Organism Toxicity Testing

The Agency has determined that the non-target organisms most likely to be exposed to the protein in transgenic cotton fields are beneficial insects feeding on cotton pollen and nectar, and soil invertebrates. Direct field census data on the abundance of invertebrates in the field were also requested, received and evaluated. The toxicity of the Cry1F/Cry1Ac proteins has been evaluated following testing of several species of invertebrates, including: adult and larval honey bees, a parasitic hymenopteran (*Nasonia*), green lacewings, lady beetles, Collembola (springtail), monarch butterfly and earthworms as well as birds, terrestrial mammals, fish, and aquatic invertebrates. Reproductive and developmental observations were also made on Collembola, honey bee and lady beetle larva maturation studies. The August 2002 SAP, however, found the green lacewing and parasitic wasp studies lacking and recommended testing of alternative species. The August 2002 SAP also suggested that additional soil degradation testing is desirable in a larger variety of soils and climatic conditions. The non-target organisms tested are chosen as representative indicators of the major groups of wildlife and based on the potential for field exposure as deduced from data on Cry1F/Cry1Ac protein expression in the plant.

The form of the test substances used in the studies for this assessment are plant material such as leaves, pollen and purified bacterially-produced Cry1F/Cry1Ac proteins, separately and in combination, incorporated into the test species diet. The October 2000 SAP provided guidance to the Agency that while actual plant material is the preferred test material, bacterially-derived protein is also a valid test substance, especially where the test animals do not consume cotton plant tissue and where large amounts of Cry protein are needed for maximum hazard dose testing. As per the OPPTS Harmonized Testing Guidelines, the adult insect studies were generally of 30 days duration or until the negative control mortality reached 20%. Larval studies were through pupation and adult emergence.

Table 2. Results of Non-target Organism and Soil Fate Studies

Guideline No	Study	Results	MRID No.

Guideline No	Study	Results	MRID No.
USEPA OPPTS 885.4150	Wild Mammal Testing, Tier I	Mammalian wildlife exposure to Cry1F/Cry1Ac proteins is considered likely; however, the Cry1F/Cry1Ac protein toxicity data for Human Health Assessment indicate that there is no significant toxicity to rodents from testing at the maximum hazard dose. Therefore no hazard to mammalian wildlife is anticipated. This study was waived upon request.	Not Applicable
885.4050	A Dietary Toxicity Study with the Northern Bobwhite Quail	The acute dietary LC_{50} value for northern bobwhite exposed to cotton meal prepared from seeds expressing Cry1F and Cry1Ac proteins for 8 days was determined to be greater than the 0.021μg Cry1F/g cotton meal and 0.012 μg Cry1Ac/g cotton meal (> 100,000 ppm diet). No adverse effects on avian wildlife are expected from incidental field exposure to WideStrike® cotton. A higher concentration and longer duration broiler study is recommended. **Acceptable**.	458084-14
885.4100	Avian Pulmonary/Inhalation Testing, Tier I,	Data not required for non-infectious active ingredients	Not Applicable
885.4200	Freshwater Fish Testing	The Fish Acute Toxicity Test, Freshwater and Marine (USEPA OPPTS 850.1075) MRID NO: 458084-13 in the table below is **Acceptable** to fulfill this data requirement.	458084-13
850.1075	Fish Acute Toxicity Test, Freshwater and Marine	The 8-day $LC_{50}$for rainbow trout is greater than 100 mg a.i./kg-diet. No mortality or sublethal effects were observed. In view of the lack of toxicity and minimal aquatic exposure, no fresh water fish hazard is expected from cultivation of WideStrike® cotton crops. **Acceptable**	458084-13
850.1010	Aquatic Invertebrate Acute Toxicity Test, Freshwater Daphnids,	In a 48-hour static test with *Daphnia magna*, there were no observed adverse effects with Cry1F and Cry1Ac in combination at respective concentrations of 510 and 2,500 μg/L. Therefore, no hazard to aquatic invertebrates is expected from incidental exposure to WideStrike® cotton pollen. **Acceptable** to fulfill the OPPTS 885.4240 data requirement	458084-12
885.4280	Estuarine and Marine Animal testing, Tier I	The Fish Acute Toxicity Test, Freshwater and Marine (USEPA OPPTS 850.1075) MRID NO: 458084-13 in the table above is Acceptable to fulfill this data requirement.	458084-13
885.4300	Nontarget Plant Studies, Tier I	Since the active ingredient in this product is an insect toxin (*Bt* endotoxin) that has never shown any toxicity to aquatic or terrestrial plants, these studies have been waived for this product upon request. Outcrossing issues are addressed below.	Not Applicable

Guideline No	Study	Results	MRID No.
885.4380	Honey Bee Larva Testing Tier I	At 1.98 μg Cry1F + 11.94 μg Cry1Ac per mL sugar water no effect on survival of larvae to adult emergence was seen. The LC50 is >4X pollen expression. Therefore no hazard to honey bee larvae and adult bee emergence is anticipated. **Acceptable**	455423-16
885.4340	Parasitic Hymenoptera Larva Testing Tier I	At 5.2 μg Cry1F + 46.8 μg Cry1Ac per mL sugar water at 10 d, no effect of limit dose with $_{LC50}$ > 13X pollen expression was seen. Minimal exposure and no hazard to parasitic Hymenoptera from Cry1F/Cry1Ac protein is expected. Testing of a species more common to cotton fields is recommended. **Acceptable**.	458084-11
885.4340	A Dietary Toxicity Study with Green Lacewing Larvae Tier I	No effect is noted at Cry protein levels expressed in pollen that would be encountered by green lacewings in the field. Because of questionable ingestion of the test material another species (e.g, minute pirate bug), which is more likely to be exposed, should be tested. **Supplemental** to testing *Orius insidiosus*	458084-10
885.4340	Adult Lady Beetle Testing. Tier I *(H. convergens)*	At 300 μg Cry1F + 22.5 μg Cry1Ac per mL sugar water, no effect of limit dose with LC_{50}> 780X Cry1F pollen expression and > 8X Cry1Ac pollen expression. Based on these results no hazard to *H. convergens* is expected when feeding on WideStrike® cotton pollen in the field. **Acceptable**	455423-15
885.4340	Collembola Chronic Dietary Toxicity Study Tier I	The combination of 709 μg Cry1F + 22.6 μg Cry1Ac per g diet and cotton leaf tissue showed no effect on adult survival and reproduction at up to 10X the anticipated field level of expression. Therefore, no hazard to decomposers represented by collembola is expected from exposure to WideStrike® cotton in the field. **Acceptable**	458084-09
OECD Guideline 207	Earthworm Toxicity Study	A 14-day study for earthworms exposed to soils treated with microbial-produced Cry1Ac and Cry 1F, individually and in combination, was performed. There were no overt signs of toxicity to earthworms exposed to soils containing nominal concentrations of Cry1F and Cry1Ac at 50x the expected worst case EEC. **Supplemental**	455807-01
885.4340	Monarch Butterfly Larval Pollen Exposure Calculation	The calculated EC_{50} >10^5 the dietary pollen exposure for Cry1F and >10X the dietary pollen exposure for Cry1Ac. The calculations indicate that young monarch larvae (at the most sensitive stage) will not be adversely affected by exposure to WideStrike® cotton. This is not a Guideline data requirement. **Supplemental**.	458084-20
Not Guideline	Insecticidal Activity Spectrum studies	The activity spectrum of Cry1F and Cry1Ac was determined for nine insect species representing three	458084-20

Guideline No	Study	Results	MRID No.
Data		orders and four families. Both Cry1F and Cry1Ac activity was restricted to lepidopteran insects. **Supplemental**.	
154-3500	Field evaluation of WideStrike® cotton exposure on non-target organisms Tier IV	The preliminary results from Tier IV field census studies. are supplemental to Tier I maximum hazard dose testing. The data do not show any WideStrike® cotton related adverse effect on non-target and beneficial invertebrate abundance in the field. **Supplemental**	458084-19
885.5200	Expression in a terrestrial Environment Tier II	The soil half-life of the plant expressing Cry1F and Cry1Ac was estimated as 1.3 days in a laboratory study with a representative soil from a cotton growing region. The Cry proteins were not detectable after 14 days. These results verify that the Cry1F and Cry1Ac proteins degrade rapidly. Additional multi year field testing is requested. **Acceptable**.	455568-01

2. Non-target Wildlife Testing and Hazard Assessment

a) Exposure Estimates

Exposure estimates for organisms directly feeding on cotton plants or plant parts containing Cry1F and Cry1Ac PIPs are based on the high-end expression for the relevant plant tissue to which a non-target organism may be exposed. High-end exposure estimates (HEEE) represent the 90% upper bound of the reported expression. Indirect exposures represent inadvertent exposures to Cry1F and Cry1Ac protein through soil, water, pollen on host plant tissue or multitrophic interactions. These exposures are expressed as Estimated Environmental Concentrations (EEC) and are conservatively calculated using high-end estimates for input parameters. Risk is characterized by comparing the exposure estimates (HEEE or EEC) to toxicity levels.

Direct feeding on plants or plant parts constitutes the primary route of exposure of organisms to Cry1F and Cry1Ac expressed in MXB-13. Plant parts subject to feeding are leaves, roots, stems, pollen and nectar. Plant pests which directly feed on cotton as their primary food source are not germane to this assessment. Organisms incidentally exposed to cotton plants or plant residues as an occasional or supplementary food source are considered non-target organisms of concern in this exposure assessment. Secondary exposure to PIP residues by tritrophic interactions may occur for predators or plant-feeding organisms.

Evaluation of protein expression concentrations and routes of exposure provide estimated levels of exposure conservatively projected to occur in the environment. Levels of Cry1F and Cry1Ac measured in tissues collected from transgenic cotton line MXB-13 are presented in **Table 3**.

Also, the HEEE are presented for tissues relevant to estimating exposure concentrations.

Table 3. Cry1F and Cry1Ac expression levels in cotton tissue.

Matrix	Mean	Standard Deviation	Min/Max Range	HEEE[a]
		*(ng **Cry1F**/mg tissue[b])*		
Young leaves (3-6 wk)	6.81	3.58	2.8-19.2	
Terminal leaves	8.19	3.5	3.0-19.5	15.05
Squares	4.88	1.8	0.97-9.9	
Flower	5.44	1.84	1.9-11.4	
Whole Plant (seedling)	14.1	5.6	8.0-28.4	
Whole Plant (pollination)	25.3	11	0.05-48.0	
Whole Plant (defoliated)	22.0	11	7.6-40.2	43.56
Root (seedling)	0.88	0.73	0.18-0.27	
Root (pollination)	0.54	0.4	0.13-1.8	
Root (defoliated)	0.51	0.2	0.26-0.87	0.90
Boll (early)	3.52	1.7	0.91-8.8	
Seed	4.13	1.11	1.4-6.6	6.31
Pollen	0.06	0.15	ND[c]-0.51	0.35
Nectar	ND	NA[d]	ND-ND	
		(ngCry1Ac/mg tissue)		
Young leaves (3-6 wk)	1.82	0.6	0.50-3.7	
Terminal leaves	1.31	0.4	0.43-2.1	2.09
Squares	1.82	0.5	0.83-3.0	

Matrix	Mean	Standard Deviation	Min/Max Range	HEEE[a]
Flower	1.83	0.4	1.1-2.8	
Whole Plant (seedling)	1.37	0.4	0.94-2.4	
Whole Plant (pollination)	1.05	0.2	0.79-1.3	
Whole Plant (defoliated)	0.6	0.2	0.31-0.92	0.99
Root (seedling)	0.17	0.06	0.06-0.27	
Root (pollination)	0.07	0.06	ND-0.15	0.19
Root (defoliated)	ND	NA	ND-0.09	
Boll (early)	0.64	0.2	0.21-1.0	
Seed	0.55	0.07	0.44-0.70	
Pollen	1.45	0.5	1.0-2.5	2.43
Nectar	ND	NA	ND-ND	

[a]High end exposure estimate (HEEE) = 90% upper bound = [mean + 1.96 x (standard deviation)]
[b]Seed, pollen and nectar are reported in a fresh weight basis; all other results are reported on a dry weight basis for lyophilized samples.
[c]ND-not detected, limit of quantitation (LOQ) = 0.15 ng/mg (pollen, root), ~ 0.05ng/mg (nectar)
[d]NA-not applicable

b) Estimated Environmental Concentrations [MRID No. 458084-20]

EEC's in soil and water matrices were calculated to conservatively represent exposure by indirect routes for comparison against tier I non-target species testing endpoints. The basis for EEC computations is expression data for MXB-13 cotton which describe relevant HEEE for Cry1F and Cry1Ac proteins in plant tissues at harvest and conservatively based models that predict concentrations in soil and water. The basis for calculation of EEC reported here is predicted biomass production and partitioning as determined for average cotton yield. From this and literature estimates of biomass production and dry matter partitioning in cotton, the HEEE for expression are converted into EEC in soil and water. The EEC in soil for Cry1F is 0.317 mg a.i./kg soil and that for Cry1Ac is 0.0196 mg a.i./kg soil.

Cotton is predominately a self-pollinated crop with some amount of cross-pollination facilitated

by bees. Lepidopteran insects are not pollinators of cultivated cotton and indirect exposure to cotton pollen is negligible.

Both target and non-target insect herbivores serve as food sources for beneficial insect predators and prey which constitute a relevant exposure route within a multitrophic context. The concentrations of Cry1Ac protein found in aphids were a minimum of 100-fold lower upwards to several thousand-fold lower than in food sources containing the Cry1Ac protein. Similarly, Lepidoptera showed reduction in Cry1Ac protein concentration in comparison to their food source but the level of reduction was less dramatic than for aphids.

c) Mammalian Wildlife Hazard Assessment [Wild Mammal Testing Tier I, USEPA OPPTS 885.4150]

Mammalian wildlife exposure to Cry1F and Cry1Ac protein is considered likely; however, the mammalian toxicology information gathered to date on Cry1F and Cry1Ac proteins does not show a hazard to wild or domesticated mammals. Microbial protein preparations of Cry1F and Cry1Ac administered to CD1 mice by oral gavage for the Human Health Assessment indicate that there is no significant toxicity to rodents from acute oral testing at the maximum hazard dose (Cry1F >600 mg a.i./kg and Cry1Ac >700 mg a.i./kg.). Therefore no hazard to mammalian wildlife is anticipated.

d) Avian Hazard Assessment [Avian Oral, Tier I, USEPA OPPTS 885.4050. [MRID No. 458084-14]]

The acute dietary toxicity of a basal avian diet fortified with cotton seed expressing the Cry1F and Cry1Ac insecticidal proteins was evaluated in young northern bobwhite (*Colinus virginianus*) for 8 days. Thirty bobwhite chicks, 10-days old, were fed a diet fortified with 10% cotton meal from cotton seeds expressing the Cry1F and Cry1Ac protein for five days. The bird feed was amended with 10% cotton seed meal from cotton seed containing nominal 0.021 μg Cry1F/g cotton seed meal and 0.012 μg Cry1Ac/g cotton seed meal. Although no mortality was observed from the two control groups, nor in birds exposed to the insecticidal proteins, clinical symptoms of wing droop, lethargy and ruffled appearances were evident in the majority of birds from both groups receiving diets amended with cotton seed meal. These clinical symptoms were attributed to markedly high levels of gossypol−8-10X the maximum allowable in animal feeds. The dietary LC_{50} value for northern bobwhite exposed to cotton meal prepared from seeds expressing Cry1F and Cry1Ac proteins was determined to be greater than the 0.021 μg Cry1F/g cotton meal and 0.012 μg Cry1Ac/g cotton meal (> 100,000 ppm diet prepared with cotton seed from transformed cotton with both Cry proteins). The no mortality concentration was >0.021 μg Cry1F/g cotton seed meal and >0.012 μg Cry1Ac/g cotton seed meal. The study is insufficient to assess hazards to avian species which may be exposed continuously to high levels of Cry1F and Cry1Ac in domestic poultry feed which normally contain 60 to 70% corn, and/or 10% cottonseed meal. However, in consideration of gossypol toxicity noted in the transgenic cottonseed and the reference control cottonseed, a six-week broiler chicken study in which 60-70% cotton meal from Cry1F/Cry1Ac-cotton is used for the diet will be an acceptable protocol to make a dietary

assessment for the two combined proteins.

A summary of an acute oral study (referenced in MRID #45808420) found the acute oral LD_{50} for northern bobwhite quail exposed to a single oral dose of Cry1F/Cry1Ac (7.9:1 ratio) to be >128 mg a.i./kg, the limit test dosage. No mortality, clinical signs of toxicity or treatment related effects were observed from exposure to the limit dose.

e) Aquatic Species Hazard Assessment

There is no evidence for sensitivity of aquatic (including endangered) species to anti-lepidopteran Cry proteins. Toxicity studies with lepidopteran-active Cry proteins on aquatic organisms show no hazard for fish or invertebrates exposed to either pollen or to bacterially expressed Cry protein. In addition, aquatic exposure from *Bt* cotton is extremely small. [The October, 2000 and August, 2002 SAP reports recommended that non-target testing be focused on species exposed to the crop being registered. Therefore, testing of aquatic species was performed primarily to satisfy the testing requirements for microbial toxins published in 40 CFR Part 158.]

i. Freshwater Fish Hazard Assessment [Fish Acute Toxicity Test, Freshwater USEPA OPPTS 885.4200. [MRID No. 458084-13]

In an eight day study, the acute dietary toxicity to the rainbow trout (*Onchorynchus mykiss*) was determined by providing a basal fish diet fortified with 100-mg a.i./kg diet mixture of Cry1F/Cry1Ac in a ratio of approximately 7.9:1. No mortality or sublethal effects were observed. The 8-day LC_{50} for rainbow trout is greater than 100 mg a.i./kg-diet. In view of the lack of demonstrated toxicity and minimal aquatic exposure, no fresh water fish hazard is expected from the proposed uses of WideStrike® cotton crops.

ii. Aquatic Invertebrate Hazard Assessment [Aquatic Invertebrate Acute Toxicity Test, Freshwater Daphnids USEPA OPPTS 850.1010 [MRID No. 458084-12]]

The acute toxicity of microbial-produced 510 μg Cry1F/L + 2500 μg Cry1Ac/L of test solution to instars of *Daphnia magna* was assessed in a 48-hour static test. No immobility or other adverse effects were seen during the study. Based on biological interpretation of the data, the 24-hour and 48-hour EC_{50}s for daphnia exposed to the Cry1F + Cry1Ac mixture were >510 μg Cry1F/L and >2500 μg Cry1Ac/L (represents a worst-case exposure of one kg of transgenic cotton pollen per liter of pond water). This rate of fortification represents 298X and >23,000X the anticipated EEC for Cry1F and Cry1Ac protein in surface water. Therefore, no hazard to aquatic invertebrates is expected from incidental exposure to WideStrike® cotton pollen.

f) Estuarine and Marine Animal Hazard Assessment [Fish Acute Toxicity Test, Marine USEPA OPPTS 885.4208. [MRID No.458084-13]]

In an eight day study, the acute dietary toxicity to the rainbow trout (*Onchorynchus mykiss*) was determined by providing a basal fish diet fortified with 100-mg a.i./kg diet mixture of

Cry1F/Cry1Ac in a ratio of approximately 7.9:1. No mortality or sublethal effects were observed. The 8-day LC_{50} for rainbow trout is greater than 100 mg a.i./kg-diet. In view of the lack of demonstrated toxicity and minimal aquatic exposure, no estuarine or marine animal hazard is expected from the proposed uses of WideStrike® cotton crops.

g) Terrestrial and Aquatic Plant Hazard Assessment [Nontarget Plant Studies, Tier I USEPA OPPTS 885.4300]

Since the active ingredient in this product is an insect toxin (*Bt* endotoxin) that has never shown any toxicity to plants, these studies have been waived for this product upon request. Outcrossing issues are addressed below.

h) Terrestrial Invertebrate Testing and Hazard Assessment

The Agency understands that routine agronomic practices have included the long term use of chemical pesticides, which have adverse effects on soil organisms, and this practice has not resulted in an accumulation of significant amounts of plant detritus in soils. Thus, Cry protein expressed in crops is expected to have less impact on these species than chemical pesticides and should not result in any increased build up of plant detritus or Cry proteins at toxic levels. Supporting this conclusion are data received by EPA that indicate that such proteins are known to degrade rapidly in field soils. Cry proteins that become bound to soil particles have been shown to be rapidly degraded by soil microbes upon elution from the soil particles. Therefore the potential for significant soil buildup and adverse effects to non-target soil organisms are not anticipated. It has been confirmed in published literature that *Bt* Cry protein released from root exudates and the biomass of *Bt* plants has no apparent effect on earthworms, nematodes, protozoa, algae, bacteria, actinomyces and fungi in soil in spite of the fact that enough detectable Cry protein is bound to soil particles to show toxicity to the target pest. These results suggest that despite its presence in soil, the Cry protein released in root exudates of some *Bt* crops, or from the degradation of the *Bt* crop biomass, is not toxic to a variety of organisms in the soil environment. It has also been reported that the same degree of *Bt* Cry protein persistence takes place in soils that have been exposed to repeated *Bt* microbial spray applications. In addition, new plants grown in *Bt* containing soil do not take up the *Bt* protein. Nevertheless, data on insects closely related to the target pest, as well as other studies to address the published data requirements for registration of microbial toxins (40 CFR §158) have been received and reviewed.

i. Single Species Laboratory Testing

The test material fed to the invertebrate species in several of the studies is purified microbial Cry1F and Cry1Ac protein in a ratio equivalent to the Cry1F and Cry1Ac proteins in whole WideStrike® cotton plants. The toxicity tests conducted with non-target arthropods indicate that no adverse effects are expected when exposed to Cry1F and Cry1Ac protein concentrations exceeding the EECs.

ii. Effects on Honey Bee Larvae [Honey Bee Testing, Tier I USEPA OPPTS 885.4380 [MRID No 455423-16]]

Honey bee larvae were exposed to sucrose solutions containing the following 3 test substances; genetically modified pollen (Cry1F), genetically modified pollen (Cry1Ac), and *Bt* with both Cry1F and Cry1Ac expression. Honey bee larvae control groups were exposed to non-genetically modified pollen, a positive control of potassium arsenate and a negative control of sucrose only. Honey bee larva survival to capping and to emergence as adults, was 90.00% in the genetically modified pollen (Cry1F) group, 76.25% in the genetically modified pollen (Cry1Ac) group and 90.00% in the group exposed to *Bt* with both Cry1F and Cry1Ac expression. All three treatment groups were not statistically different from the negative control (sucrose only) which had a survival of 95% to capping and 93.75% to adult emergence. The positive control exhibited only 45% survival to capping and adult emergence. Based on these data, the LD_{50} for honey bees is > 2 mg cotton pollen expressing the Cry1F or Cry1Ac proteins and >1.3 mg Cry1F delta-endotoxin plus >8.5 mg ± 5% of Cry1Ac delta-endotoxin from bacterial sources per 100 mL 30% sucrose. These results indicate honey bee mortality as evaluated by capping and adult emergence was not affected by exposure to any of the test substances, therefore no honey bee hazard is expected from the proposed uses of WideStrike® cotton crops.

iii. Parasitic Hymenoptera Hazard Assessment [Parasitic Hymenoptera Testing Tier I USEPA OPPTS 885.4340 [MRID No 458084-11]]

In a limit test, the study authors concluded that prepared diets containing 5.2 μg Cry1F/mL, 46.8 μg Cry1Ac/mL, or 5.2 μg Cry1F + 46.8 μg Cry1Ac/mL did not affect the mean mortality of the parasitic hymenopteran *Nasonia vitripennis* after ten days of exposure. Surviving larvae in all groups were generally normal in appearance and behavior. Based on this study, the dietary LC_{50} were: > 5.2 μg Cry1F/gram of diet, >46.8 μg Cry1Ac/gram of diet, >0.52 μg Cry1F + 4.68 μg Cry1Ac/gram of diet, >5.2 μg Cry1F + 46.8 μg Cry1Ac/gram of diet, and > 5.2 μg heated Cry1F Ac + 46.8 μg heated Cry1Ac per gram of diet. A 40% mortality observed in the Cry1F + Cry1Ac group at 32X the EEC is less than a LC_{50} at 32X the possible field exposure (EEC). The EPA level of concern for terrestrial wildlife is a LC_{50} at less than 5X the field exposure (EEC/LC_{50} = RQ > 0.2). Therefore since the LC_{50} in this study is greater than 32X the EEC, no hazard to parasitic Hymenoptera is expected at field exposures which are minimal to nonexistent.

iv. Green Lacewing Larvae Hazard Assessment [Green Lacewing testing Tier I. USEPA OPPTS 885.4340 [MRID No 458084-10]]

In tests of dietary toxicity (mean survival to pupation) of Cry1F, Cry1Ac, and Cry1F + Cry1Ac mixtures to green lacewing larvae (*Chrysoperla carnea*), the dietary LC_{50}s were: > 5.2 μg Cry1F/gram of diet, >46.8 μg Cry1Ac/gram of diet, >0.52 μg Cry1F + 4.68 μg Cry1Ac/gram of diet, >5.2 μg Cry1F + 46.8 μg Cry1Ac/gram of diet, and > 5.2 μg heated Cry1F Ac + 46.8 μg heated Cry1Ac per gram of diet. Mortality was increased and pupation was affected in the Cry1F/Cry1Ac at 32X the concentration found in pollen. (LC_{50} > 14X pollen expression). No effect is noted at Cry protein levels expressed in pollen that would be encountered by green

lacewings in the field.

However, the appropriateness of the methodology for the green lacewing acute toxicity study is questionable. In addition, green lacewings are difficult to test in the laboratory because of a high rate of mortality. The August 2002 FIFRA Scientific Advisory Panel (SAP) also noted concerns regarding the green lacewing methodology. The SAP questioned whether the green lacewings are ingesting the Cry protein that is coated around moth eggs in a diet. Since green lacewing has piercing-sucking mouthparts, they may not be exposed to the protein on the external surface of the egg diet. Finally, the SAP questioned the appropriateness of testing green lacewing and recommended testing an alternate natural enemy such as the minute pirate bug (*Orius insidiosus*). Therefore, an additional Tier 1 non-target insect test with the minute pirate bug should be conducted with the Cry1F and Cry1Ac proteins. *Orius* typically occur in fields as egg predators and they typically feed on pollen. Therefore, a laboratory study should be conducted feeding *O. insidiosus* both pollen and purified protein in diet. Feeding *O. insidiosus* Cry proteins in diet will allow for a test at the maximum hazard dose; whereas, feeding *O. insidiosus* pollen expressing the Cry proteins will provide an evaluation of potential effects from actual exposure scenarios.

v. Lady Beetle Hazard Assessment [Lady Beetle Testing. Tier I. USEPA OPPTS 885.4340 [MRID No. 455423-15]]

Adult Lady beetles (*Hippodamia convergens*) were exposed to either a single dietary dose of 300 μg a.i./mL of Cry1F, a single dose of 22.5 μg a.i./mL of Cry1Ac or a combined dose of 300 μg a.i./mL of Cry1F plus 22.5 μg a.i./mL of Cry1Ac as a mixture with sugar water. Four replicates of 25 beetles each were used for treatment and control groups which were observed for mortality and clinical changes until the negative control mortality exceeded 20% on day 15 of the test. Cumulative mortality and signs of toxicity observed in the treatment groups were used to calculate the dietary LC_{50}. The dietary LC_{50} was greater than 300 μg a.i./mL for Cry1F, greater than 22.5 μg a.i./mL for Cry1Ac, and greater than the combined dose of 300 μg a.i./mL for Cry1F plus 22.5 μg a.i./mL for Cry1Ac. This study demonstrates that lady beetles will not be adversely affected by the proposed uses of WideStrike® cotton.

vi. Collembola Hazard Assessment [Collembola Testing. Tier I. USEPA OPPTS 885.4340 [MRID No. 458084-09]]

The chronic effects of Cry1F and Cry1Ac were assessed on Collembola (*Folsomia candida*) using microbially-derived Cry1F and Cry1Ac added to brewers yeast. In 28-day dietary toxicity tests, 709 mg Cry1F/kg of diet or 702 mg Cry1F + 22.6 mg Cry1Ac per kg of diet did not adversely affect mortality or reproduction of Collembola. The dietary concentration of approximately 709 mg a.i. of Cry1F /kg diet and 22.6 mg a.i. of Cry1Ac /kg diet represents >1,100-fold higher levels than those anticipated in the field. Diets containing 22.6 mg Cry1Ac/kg alone did not affect mortality but decreased reproduction by up to 45%; however, the toxicity was attributed to impurities in the Cry1Ac test material. Lyophilized Cry1Ac cotton leaf at 5% or 50% of the diet had no adverse effect on mortality or reproduction. The combination of Cry1F and Cry1Ac showed no effect at up to 10X the anticipated field level of expression. Therefore, no hazard to

decomposers represented by Collembola is expected from exposure to WideStrike® cotton in the field.

This study adequately addresses potential concerns for Cry1F and Cry1Ac protein expressed in transgenic cotton to Collembola (*Folsomia candida*), a representative of beneficial soil insect species. The results of this study demonstrate that Cry1F and Cry1Ac proteins found in transgenic cotton pose no hazard to soil inhabiting Collembola species and by inference to other beneficial non-lepidopteran soil insects. It is notable that recent recommendations by the SAP (March, 2001) are that invertebrates of different orders than those known to be affected by the Cry protein in question need not be tested.

vii. Earthworm Hazard Assessment [Acute Toxicity to Earthworms. OECD Guideline 207 [MRID NO. 455807-01]]

A 14-day limit dose study was conducted on earthworms exposed to soils treated with microbial-produced Cry1Ac and Cry 1F, individually and in combination. There were no overt signs of toxicity to earthworms exposed to soils containing nominal concentrations of Cry1F and Cry1Ac at 50x the expected worst case EEC [this represents concentrations which are 792X and 5479X higher than the expected EEC for incorporation of defoliated cotton plants into the top 15 cm of soil.] The 14-day LC_{50}s were >247 mg a.i./kg for Cry1F; >107 mg a.i./kg for Cry1Ac, and > 247 mg a.i./kg Cry1F + >107 mg a.i./kg Cry1Ac in the test with the two proteins combined. These data show that no adverse effects to earthworms are expected in fields growing WideStrike® cotton. [This study was rated as Supplemental. The study was conducted at nominal test material concentrations]

Earthworm feeding studies submitted to the Agency for all of the registered Cry proteins demonstrate that the Cry proteins are not toxic to earthworms at the worst case environmental concentration. Recently published data show that the earthworms ingest the *Bt* Cry proteins with the soil without harmful effects. These reports also show that there were no significant differences in the percent mortality and weight of earthworms after 40 days in soil planted with *Bt* or non-*Bt* corn, in fallow fields, or after 45 days in soil amended with biomass of *Bt* or non-*Bt* corn or not amended. The *Bt* Cry protein was shown to be present in both the casts and guts of the worms.

The reviewed data show that no adverse effects to earthworms are expected in fields growing WideStrike® cotton.

viii. Monarch Butterfly Risk Assessemnt [Non-target insect testing. Tier I. USEPA OPPTS 885.4340 [MRID No. 458084-20]]

Studies conducted by Hellmich, et al. (Proc. Nat. Acad, Sci. 98 [21]: 1925-11930) were used to show that the density of cotton pollen on milkweed leaves (11 grains of MXB-13 pollen per cm^2) is 10X less than the minimum pollen density required to elicit subchronic or developmental

effects on monarch butterfly larvae. The EC_{50} $>10^5$ the dietary pollen exposure for Cry1F and > 10X the dietary pollen exposure for Cry1Ac. The calculations indicate that young monarch larvae (at the most sensitive stage) will not be adversely affected by exposure to WideStrike® cotton pollen expressing Cry1F and Cry1Ac proteins in the field.

ix. Insecticidal Activity Spectrum Study [Susceptible insect spectrum of Cry1F and Cry1Ac Insecticidal Crystal Proteins. Non-Guideline studies. [MRID No. 458084-20]]

The insecticidal activity spectrum of Cry1F and Cry1Ac was determined for nine insect species exposed to microbially-expressed Cry1F and Cry1Ac in artificial-diet studies. The insects represent taxonomically diverse cotton pests including three orders (Lepidoptera, Heteroptera and Coleoptera) and four families (Miridae, Curculionidae, Noctuidae and Gelchiidae). Insects evaluated were:

tobacco budworm (TBW) - *Heliothis virescens*
cotton bollworm (CBW) - *Helicoverpa zea*
beet armyworm (BAW) - *Spodoptera exigua*
western tarnished plant bug (WTPB) - *Lygus hesperus*
boll weevil (BW) - *Athonomus grandis*
soybean looper (SBL) - *Pseudoplusia includens*
fall armyworm (FAW) - *Spodoptera frugiperda*
cabbage looper (CL) - *Trichoplusia ni*
pink bollworm (PBW) - *Pectinophora gossypiella*
cotton aphid (CA) - *Aphis gossypii*

Both Cry1F and Cry1Ac activity was restricted to lepidopteran insects lending support to the contention that the combination of two Cry proteins did not expand the insect host range. These data also support the observations that *Bt* Cry proteins have a very specific and narrow range of target species.

xi. Field Evaluation of WideStrike® Cotton Effects on Invertebrates [Non-target Beneficial Arthropod Field Survey (MRID No. 458084-19). [Supplemental, but additional studies were not required in the terms and conditions of registration.]

The submitted field monitoring studies substantiate the Tier I single species data showing a lack of adverse non-target invertebrate effects of MXB-13 cotton. The beneficial arthropods present in field plots of MXB-13 cotton were compared with those in field plots of non-transgenic cotton with comparable genetics as well as those with and without insecticide application at locations in Louisiana and Arizona. Preliminary results show no adverse effect of MXB-13 on the numbers of insects from over 50 taxa monitored using scouting, whole plant sampling and sweeps. Synthetic (chemical) insecticide treatment, however, reduced the population of some taxa of non-target arthropods at certain times of sampling.

In 2002, field surveys using sweep net and sticky trap sample methods were conducted at two

locations to evaluate potential effects on non-target beneficial arthropods of the MXB-13 pyramided cotton line (Cry1F/Cry1Ac). Analyses of all data collected at Winnsboro, LA revealed that there were no adverse effects of MXB-13 on non-target beneficial arthropods. The MXB-13 with no chemical insecticide treatment for Lepidoptera showed significantly higher seasonal survey counts for beneficial Heteroptera in sweeps and for lady beetle adults in leaf sampling while demonstrating effective control of bollworm larvae. Likewise, field studies conducted at the Maricopa Agricultural Research Center in Arizona showed no apparent negative effects to non-target organisms from the MXB-13 cotton line to the nearly 200 arthropods examined from sweep net collections and the 143 arthropods examined from aerial traps. Several insect groups were significantly more numerous in the MXB-13 plots than in the control plots sprayed with chemical insecticides for Lepidoptera. This study was conducted for one year using two sample methods in small plots. Additional field studies were not recommended by the 2004 SAP.

The Agency is reviewing the available field studies as data supplemental to the maximum hazard dose single species laboratory testing, but nonetheless useful for a short range assessment of non-target invertebrate abundance in Cry protein expressing crop plots. Since the endpoint for field census studies has not been determined, it is difficult to determine how large the fields should be, how many replications are needed and how many samples per plot are needed to achieve appropriate statistical power. If Tier I maximum hazard dose single species laboratory studies show a hazard, intermediate field or semi-field studies between laboratory and full-scale field studies should be conducted.

2. Environmental Fate

a. Soil Degradation Studies [Expression in a terrestrial Environment, Tier II (Environmental Fate) [MRID No. 455568-01]]

The soil half-life of the plant expressed Cry1F and Cry1Ac was estimated as 1.3 days in a laboratory study with a representative soil from a cotton growing region (Wayside, Mississippi). Soil fortification rates for the study were 0.072 mg a.i. Cry1Ac and 0.853 mg a.i. Cry1F per kg of oven dry soil. These levels represent approximately 3.2 X the EEC for incorporation of defoliated whole plants of MXB-13 into the top 15-cm of soil.

The soil degradation study was conducted with cotton leaf tissue expressing the Cry1F and Cry1Ac insecticidal proteins (2.87 μg/g Cry1Ac and 34.1 μg/g Cry1F). Lyophilized cotton leaf tissue was mixed with soil, incubated under standard laboratory conditions and sampled for bioassay at various intervals. Insect bioassay was conducted to measure degradation via biological activity by applying aqueous-agar mixtures of soil samples to the top of artificial diet and allowing neonate tobacco budworms (*Heliothis virescens*) to feed on the treated media. Test concentrations of Cry1Ac/Cry1F in the surface diets were 0.0762, 0.229, 0.686, 2.06, 6.17, 18.5,

55.6 and 167 $\mu g/cm^2$. Mortality and insect weight data were collected from the insect bioassays. Growth inhibition (GI_{50}s-concentration estimated to reduce growth by 50%) was used to estimate the potency of each sample. Based on the increase seen in GI_{50}s over time, the half-life of the Cry1Ac/Cry1F proteins in a representative cotton soil was 1.3 days under laboratory conditions, indicating a rapid decay rate in soil. The Cry proteins were not detectable after 14 days.

Soil organisms may be exposed to Cry proteins by exposure to roots, incorporation of above ground plant tissues into soil after harvest, or by pollen deposited on the soil. Root exposure may occur by feeding on living or dead roots or, theoretically, by ingestion or absorption after secretion of the Cry protein into the soil. In addition, some evidence suggests that Cry proteins, while bound to some soil components, e.g. clays and humic acids, are recalcitrant to degradation by soil microorganisms, but without eliminating their insect toxicity. Several factors influence either the affinity of binding or the rate of degradation. In particular, pH near neutrality generally substantially increases degradation. These issues are being evaluated on a case-by case basis by environmental fate studies designed to determine the rate of Cry protein degradation over sufficiently long periods to assure an accurate assessment of degradation in agricultural soils.

Because the Agency believes that additional studies would be useful in completing the database for long term effects assessment, it is requesting additional supplementary studies regarding Cry1F/Cry1Ac cotton protein degradation in soil.

b. Effects on Soil Microorganisms

Published studies performed by the EPA Office of Research and Development on the impact of transgenic Cry cotton and other plants indicate that adverse effects on soil microorganisms are unlikely. No effects have been seen due to the protein itself, and only a minimal, transient increase observed in soil microbes has been attributed to the transgenic cotton plant tissue rather than the Cry protein expressed in that tissue. No adverse effects have been observed in a similar season long field study with Cry3A potato.

c. Horizontal Transfer of Transgenes from *Bt* Crops to Soil Organisms

The Agency has evaluated the potential for horizontal gene transfer (hgt) from *Bt* crops and has considered possible risk implications if it occurred. Several experiments published in the scientific literature have been conducted to assess the likelihood of hgt, and have been unable to detect gene transfer under typical field conditions. Hgt has only been detected under conditions designed to favor transfer. In addition, the genes that have been engineered into the *Bt* crops are mostly found in, or have their origin in, soil inhabiting bacteria. Soil is also the habitat of anthrax, tetanus and botulinum toxin producing bacteria. Transfer of these genes or toxins to other microorganisms or plants is not known to occur. Therefore, the Agency concluded that hgt is at most an artificial event, and the traits engineered into the *Bt* crops are already present in soil bacteria or are unlikely to have selective value for soil microorganisms. In considering these data the Agency further concludes that there is no significant risk from hgt from the transgenes found in Cry1F/Cry1Ac cotton.

d. Gene Flow and Weediness Potential

The Agency has reviewed the potential for gene capture and expression of the Cry proteins in cotton by wild or weedy relatives of cotton in the United States, its possessions or territories. There is a possibility for gene transfer in locations where wild or feral cotton relatives exist. Therefore, EPA requires stringent sales and distribution restrictions on *Bt* cotton within these areas to preclude outcrossing or hybridization from the crop to sexually compatible relatives (see Section III. Terms and Conditions of Registration). There are only three areas in the United States and its territories wherein cultivated cotton has the opportunity to outcross to wild or feral species which are genetically compatible: (1) southern Arizona, (2) Hawaiian islands, and (3) southern Florida. *G. thurberi* (Arizona Wild Cotton), is present in the elevated regions of Arizona and does not grow in areas of commercial cotton production. *G. thurberi* is a diploid and produces sterile, triploid progeny when crossed with the tetraploids *G. hirsutum* or *G. barbadense*. In the very south of Florida, feral *G. hirsutum* exists in apparently self-sustaining populations. Since these would readily cross with cultivated cotton, sale of *Bt*-Cotton is restricted south of Interstate 60. There is currently no commercial cotton production in the southern part of Florida. Evidence from germplasm collections indicates that feral *G. barbadense* and possibly *G. hirsutum* exist in the U.S. Virgin Islands. There is presently no production of commercial cotton in either of these places, hence, outcrossing is not an issue. For a detailed review of the Agency's assessment of the potential for gene capture and expression of Bt endotoxins by wild or weedy relatives of cotton in the U.S., its possessions or territories, see the EPA Biopesticides Registration Action Document (BRAD) for the *Bacillus thuringiensis* (*Bt*) Plant-Incorporated Protectants, dated October 15, 2001.

e. Endangered Species Considerations

Based on the Cry1F/Cry1Ac (WideStrike®) cotton protein toxicity and exposure data reviewed there will not be a "may effect" situation for threatened and/or endangered mammals, birds, plants and aquatic species. A comparison of the county-level distribution of endangered lepidopteran species relative to cotton producing counties in the US indicate that only the Kern primrose sphinx moth (*Euproserpinus euterpe*) is know to occur in a cotton producing county. However, cotton is not a host plant for this species nor do host-range considerations place habitat in or near cotton fields. The Kern primrose sphinx moth is the only endangered lepidopteran taxa known to occur in counties where cotton is grown.

An examination of the threatened and/or endangered bird and bat species shows that their breeding habitats are mostly non-agricultural. Insectivorous bats do not prey on larvae. They rely on flying insects. Taking these, and other pertinent issues into consideration, it becomes apparent that reduction in the target pests of cotton would not have an effect on the food source of threatened and/or endangered birds and bats. Of those that do encroach on agricultural fields, and in the rare instances where these species may feed on the target pests, the reduction in the pest species will merely cause them to rely on other plentiful insects as a source of food. Submitted

and published field data reviewed in this document show that a wide variety of insects remain abundant in Cry protein crop fields as opposed to non-*Bt* fields when conventional insect pest control practices are used. Therefore, the data show that *Bt* crops should actually be beneficial to bird and bat populations.

The reviewed non-target data confirm the expectation that WideStrike® cotton will have no adverse effect on threatened and/or endangered species listed by the US Fish and Wildlife Service, including mammals, birds or terrestrial and aquatic plants and invertebrate species. Therefore, no consultation with the USFWS is required under the Endangered Species Act

3. Environmental Assessment Summary

From all of the required and voluntarily developed indicator and host range species test data on WideStrike® cotton, including the supplementary field data, the Agency concludes that the levels of Cry1F and Cry1Ac protein in cotton will not pose unreasonable adverse effects to cotton agroecosystem flora and fauna. Available data also indicate that there should be minimal short term accumulation of Cry1F and Cry1Ac protein in agricultural soil. In addition, no adverse effects on endangered and threatened species listed by the US Fish and Wildlife Service are expected from the proposed WideStrike® cotton registration.

Incidental exposure to sensitive larval stages of a non-target butterfly or moth to Cry1F or Cry1Ac may occur if MXB-13 pollen is present on host plants and is consumed; however, the likelihood of such exposure is remote due to the insignificant outflow of pollen from cotton and the presence of other food sources which occur near cotton fields; thus, there is negligible risk from cropping of MXB-13. In excess of 300 different species of beneficial insects are know to inhabit cotton fields. Common arthropod predators and parasites of cotton fields represent orders that are insensitive to the Cry1 proteins. Additionally, these beneficial organisms are predominately predators and parasites and only in a few instances are plant product consumers. Therefore, direct risks to beneficial insects from exposure to Cry1F and Cry1Ac expressed in MXB-13 are negligible. Risk from indirect exposure through tritrophic feeding on insect host/prey is also negligible due to the low levels of exposure anticipated in comparison to effect levels shown in testing of surrogates.

Analysis of effect levels (selectivity and activity on non-targets) and exposure (exposure routes, concentrations and habitat for taxa of concern) indicates negligible ecological risks are posed by cropping of MXB-13 cotton expressing Cry1F and Cry1Ac PIPs.

At present, the Agency is aware of no identified significant adverse effects of Cry1F and Cry1Ac proteins on the abundance of non-target beneficial organisms in any population in the field, whether they are pest parasites, pest predators, or pollinators. Field census data submitted to the Agency show minimal to undetectable changes in the beneficial insect abundance or diversity. In cotton fields densities of predatory and non-target insects are generally higher on *Bt* crops than non-*Bt* crops primarily because the *Bt* crops are not subjected to the same number of applications of nonspecific pesticides. In genera invertebrate abundance studies in *Bt* crop fields do not show

a shift in biodiversity, except in cases where the predators are dependent on the pest insect as prey. In contrast, treatment with chemical pesticides, when studied, had significant effects on the total numbers of insects and on the numbers within the specific groups. To date the available field test data show that compared to crops treated with conventional chemical pesticides, the transgenic crops have no detrimental effect on the abundance of non-target insect populations. Additional field studies were not recommended by the 2004 SAP.

The Agency believes that cultivation of WideStrike® cotton may result in fewer adverse impacts to non-target organisms than result from the use of chemical pesticides. Under normal circumstances, WideStrike® cotton requires substantially fewer applications of chemical pesticides. This should result in fewer adverse impacts to non-target organisms because application of nonspecific conventional chemical pesticides is known to have an adverse effect on non-target beneficial organisms found living in the complex environment of an agricultural field. Many of these beneficial organisms are important integrated pest management controls (IPM) for secondary pests such as aphids and leafhoppers. The overall result of cultivation of cotton expressing Cry proteins is that the number of chemical insecticide applications for non-target pest control is reduced for management of multiple pest problems.

The movement of transgenes from WideStrike® cotton into weeds and other crops in the U.S., its possessions or territories has also been considered. The fate of Cry1F and Cry1Ac proteins in soils and indirect effects on soil biota has also been evaluated. Test data show that most of the Cry protein deposited into soil is quickly degraded, although a residual amount may persist in biologically active form for a longer period of time. It is also reported that detectable *Bt* Cry protein persistence exists in soils that have been exposed to repeat *Bt* spray applications. Limited data do not indicate that Cry proteins have any measurable effect on microbial populations in the soil. Horizontal transfer of genes or toxins from transgenic plants to soil bacteria has not been demonstrated. Published studies of *Bt* Cry protein in soil show no effect on bacteria, actinomyces, fungi, protozoa, algae, nematodes, springtails or earthworms. In addition, a new crop planted in *Bt* Cry protein containing soil does not take up the *Bt* protein.

4. Conclusions

This assessment finds no hazard to the environment at the present time from cultivation of Cry1F and Cry1Ac protein expressing cotton for a time-limited conditional registration period.

5. Supplemental Data Needed for Long Term Environmental Hazard Assessment

The Agency has sufficient information to believe that there is no hazard from the proposed uses of WideStrike® cotton to non-target wildlife, aquatic and soil organisms. However, the Agency is requesting additional, primarily long term effects data. The supplementary studies would provide additional weight to support the Agency's conclusions. Therefore, the Agency is requesting the following data **(Table 4)** to ascertain any possible adverse environmental effects from long term use of this product, as well as testing on more appropriate non-target invertebrates found in cotton

fields. The Agency does not believe that this data requirement was reasonably foreseeable by the applicant at the time of application.

Table 4. Supplemental data requested

Testing Category	Type of Data
Avian chronic exposure testing	The submitted avian dietary toxicity data are not sufficient to make a chronic avian hazard assessment from repeated exposure(s) to higher doses of Cry1F/Cry1Ac cotton. A six week broiler dietary study is needed to assess hazard to wild and domesticated fowl from chronic exposure to Cry1F/Cry1Ac protein. If necessary, Cry1F/Cry1Ac corn may be used in the study.
Non-target insect more appropriate for cotton fields	Conduct a maximum hazard dose laboratory toxicity test with *Orius insidiosus* (minute pirate bug).
Soil fate/terrestrial expression studies	Additional long range soil persistence field studies should be conducted.

E. Insect Resistance Management [MRID#s 45808415, 45808407, 45808417, 45808418, 46071901)]

Dow AgroSciences has provided an "acceptable" IRM plan for WideStrike®-protected cotton. They have provided a detailed analysis of the scientific basis for the product durability plan (insect resistance management strategy) and the practical implementation of the durability plan.

1. Target Pests and Perceived Risk of Resistance to *Bt* cotton

Dow AgroSciences has provided an adequate discussion of the target pests of WideStrike® cotton and the perceived risks of adaptation to *Bt* cotton, such as WideStrike® cotton. WideStrike® cotton has been shown to have effective control against the eight cotton insect pests evaluated: tobacco budworm (TBW), *Heliothis virescens* (F.); cotton bollworm (CBW), *Helicoverpa zea* (Boddie); pink bollworm (PBW), *Pectinophora gossypiella* (Saunders); beet armyworm (BAW), *Spodoptera exigua* (Hubner); southern armyworm (SAW), *Spodoptera eridania* (Stoll); fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith); soybean looper (SBL), *Pseudoplusia includens* (Walker); and cabbage looper (CL), *Trichoplusia ni* (Hubner). EPA agrees that the product durability plan should focus on the three primary, most economically and environmentally damaging lepidopteran pests: TBW, CBW, and PBW. Any plan that focuses on TBW, CBW, and PBW should be adequate to maintain susceptibility in secondary pests, such as FAW, BAW, SAW, SL, and CL.

Resistance risk is considered to be moderate for TBW, CBW, PBW, and BAW and low for FAW, SAW, CL, and SL. The resistance consequence is high for TBW, CBW, and PBW; moderate for BAW; and low for FAW, SAW, CL, and SL. A number of factors drive the risk of resistance: host range, geographic range, overwinter range, pest status in US cotton, and dose (whether high

or not). These factors will be taken into consideration separately in this review.

Efficacy data was provided by Dow, Pellow et al. (2002); MRID# 45808407. The results of 19 evaluations in efficacy trials from 2001 to 2002 indicate that Cry1F/Cry1Ac transgenic cotton line MXB-13 provided effective control against the eight cotton insect pests evaluated: TBW, CBW, PBW, BAW, SAW, FAW, SBL, and CL. That is, in all trials and for all insect pests evaluated, MXB-13 (sprayed and unsprayed) provided as good or better control when compared to the sprayed or unsprayed non-transgenic control line, PSC35 (the recurrent parent for both the Cry1F and Cry1Ac transgenic cotton events).

Results of five trials over a two-year span indicate MXB-13 provides a high level of control of TBW. MXB-13 was also shown to effectively control CBW. The level of control is at least equal to, and in many cases far superior, to optimum chemical spray programs used during ideal environmental conditions. Results also indicate that MXB-13 surpassed the effectiveness of chemical spray programs under non-ideal environmental conditions such as sustained periods of rain. Efficacy against TBW was demonstrated in both the early fruit development stage and in the late season boll maturation stage.

MXB-13 was shown to have excellent control of PBW with no measurable boll infestation compared with 23-75% for the non-transgenic control variety. In both field trials and bioassays, MXB-13 was effective at controlling various armyworm species including BAW, SAW, and FAW. In addition, data from field trails indicate that MXB-13 controls two species of loopers: SBL and CL

2. Cry1F and Cry1Ac PIPs Mode of Action

Dow AgroSciences has provided adequate information to describe the potential receptor binding patterns of Cry1Ac and Cry1F in TBW and CBW. TBW and CBW binding studies involving Cry1F and Cry1Ac indicate that each protein has unique binding sites and share one binding site. Each protein may have at least two, and perhaps as many as six binding sites. As Dow AgroSciences notes, incomplete shared binding is expected to lead to incomplete cross-resistance when resistance is mediated by receptor changes. A single mutation in a gene that codes for a receptor that can bind both Cry1Ac and Cry1F will not prevent binding of either Cry1F or Cry1Ac, singly, and thus will not allow high survival of the insect bearing two copies of it. Multiple genetic mutations are likely to be needed for high survival of cross-resistant insects.

No PBW receptor-site binding information regarding Bt toxins was provided by Dow AgroSciences. However, there is some published literature. Karim et al. (2000) examined the receptor binding properties of Cry1Aa, Cry1Ab, Cry1Ac, and Cry2Aa Bt toxins to PBW and CBW midgut epithelial membranes. Both Cry1Ab and Cry1Ac toxins showed saturable, high affinity binding to PBW and CBW brush border membrane vesicles. Cry2Aa and Cry1Aa toxins bound to BBMVs with low binding affinity, but with high binding site concentration. Saturation binding data correlated with toxicity in PBW. That is, the most potent toxins, Cry1Ac and Cry1Ab, showed high affinity saturable binding. Heterologous competition binding assays to

investigate binding site cross reactivity showed that Cry1Aa, Cry1Ab, and Cry1Ac recognize the same or common binding sites on PBW and CBW midgut epithelial membranes which is different from Cry2Aa. Ligand blot data showed that Cry1Ac binds to a major 120-kDa BBMV protein in PBW and several proteins, 120 kDa, 140 kDa, and 155 kDa in CBW. Cry1Ab binds to a major 201 kDa BBMV protein in PBW and a 170 k-Da BBMV protein in CBW.

Results from the Dow AgroSciences cotton-insect-pest susceptibility study examining the relative sensitivities of six cotton-feeding insects to the purified Cry1F (synpro) toxin showed that PBW was essentially insensitive to the toxin (Herman and Young, 1999; MRID# 45542307). This suggests that there are few, if any, binding receptors for Cry1F in the PBW midgut. Cross-resistance through modification of binding site receptors of Cry1Ac and Cry1F would therefore not be realistic.

3. Pest Adaptation Factors

a. Dose and Functional Dominance

Insect resistance management centers on reducing the mean (population wide) selective differential between insects carrying one copy of an allele for adaptation (resistance) at a given locus (*i.e.* RS heterozygotes) and those carrying no such alleles (SS homozygotes). This can be done by expressing PIPs at a dose that is expected to minimize the survival and fitness of heterozygotes. The level of PIP expression in the plants determines the fitness of SS insects in the *Bt* field, and indicates a range for the expected fitness of RS insects in the *Bt* field. Doses that cause high mortality of susceptible insects will also cause high mortality of heterozygous insects unless the R allele is dominant. At doses that cause low to moderate mortality of susceptible insects, heterozygous insects are expected to have a lower mortality, even if the R allele is nearly recessive. Because loss of binding is expected to be the primary mechanism of resistance, it is expected that R-allele conferring adaptation to *Bt* PIPs will be incompletely to completely recessive. If one copy of the gene codes for the normal receptor, then some receptor binding will still occur and some mortality will result. Therefore, a high dose leads to the expectation that adaptation will be functionally recessive, with little difference between SS and RS mortality.
Two EPA Scientific Advisory Panels (SAPs) in 1998 and 2000 concluded that for lepidopteran-active PIPs, a high dose is expected to kill a high proportion of heterozygotes. Based on SAP recommendations, high dose is defined as 25 times the dose required to kill 99% of susceptible insects (SAP 1998, 2000). The SAP recommended five imperfect methods for demonstrating high dose and indicated that at least two of them should be used to demonstrate a high dose. Three such methods were employed by Dow AgroSciences to demonstrate that 99.9% of insects in the field were killed by the dose expressed in MXB-13 that the dose in MXB-13 was sufficient to cause high mortality of instars that are around 25 times more tolerant of the PIP than are neonates (the later instar serves as a surrogate for the heterozygote), and that 25-fold dilution of plant tissue in artificial diet also caused high mortality of neonates. Season long expression of both PIPs in MXB-13 (WideStrike® cotton) is advantageous since heterozygote survival will remain low for all generations. Dow's expression data (MRID# 45808408) confirms that both

PIPs are expressed consistently at high levels throughout the growing season.

High dose data for the key targets pests, TBW, CBW and PBW, are summarized below. Additional pests controlled by this product are BAW, FAW, SAW, CL and SL. Further testing is expected to show efficacy against other minor lepidopteran pests.

1) TBW

Dow AgroSciences submitted a study investigating the high dose of MXB-13 cotton against TBW (Blanco et al. 2002; MRID# 45808417). Dow AgroSciences employed two laboratory-based and one field-based method to demonstrate high dose of MXB-13 against TBW. Because MXB-13 expresses two insecticidal proteins, (Cry1Ac and Cry1F) and because the expected durability of a pyramid of two proteins is in part dependent on the dose of the individual proteins, it is important to investigate the dose of each protein.

Three methods (two laboratory and one field) outlined by USEPA's Scientific Advisory Panel (SAP) (See 1998 and 2000 SAP reports) were used to demonstrate that Dow AgroSciences' transgenic cotton line MXB-13 expresses a high dose of two *Bt* insecticidal proteins, Cry1F and Cry1Ac, to control TBW larvae. This dose is high enough to kill nearly all susceptible TBW, and therefore, is expected to cause low survival of neonates heterozygous for resistance alleles. Using Methods 1 and 2 (SAP 1998), MXB-7 is assumed to express a high dose of Cry1Ac for control of tobacco budworm. Using Methods 1 and 2 (SAP, 1998), MXB-9 expresses a not quite high dose of Cry1F for control of TBW. That is, the Cry1Ac component of the pyramid in MXB-13 is by itself a high dose, while the Cry1F component in MXB-13 is not. Methods 1 and 2 both show that the pyramid, MXB-13, produces a high dose to control TBW. Although Cry1F expression is not quite a high dose, neonate mortality is quite high, >90% based on results from Method 1 and >83% based on results from Method 2. The field experiments (Method 3, see SAP, 1998) support that MXB-13 expresses a high dose against TBW. No larvae, other than 3 neonates compared to 679 larvae from the same sampling regime in the non-*Bt* control plots, were found, a greater than 99.5% difference. There may be less certainty in determining a high dose for TBW using Method 2 than in using Methods 1 and 3 because of the variability associated with using leaf tissue. No statistical analysis was performed to quantitatively compare the data. Based on all of the data, MXB-7 and MXB-13 express a high dose of Cry1Ac and Cry1Ac combined with Cry1F, respectively. MXB-9 expresses a not quite high dose of Cry1F for control of TBW. It is highly likely that resistance to MXB-13 will be functionally recessive, and thus evolve only very slowly in the presence of a structured refuge.

2) PBW

Cry1F is thought to not contribute significantly to the mortality of PBW in the field. Field efficacy trials showed no larvae developing to third instars from 3,450 boll entry holes in WideStrike® (Pellow, 2002; MRID# 45808407). Likewise, data from cotton line MXB-7 (which expresses only Cry1Ac) indicated that a single, third instar was found from 6,800 boll entry holes. Thus, it is expected that the mortality of PBW in the field is 99.99% and therefore the resistance

to WideStrike® is very likely functionally recessive, with very low survival of insects carrying single copies of alleles for adaptation.

The two methods that were chosen by Dow AgroSciences demonstrate that the MXB-13 cotton plants expressing Cry1Ac and Cry1F meet the definition of high dose for PBW as described by the SAP panels (SAP 1998, 2000). They kill >99.9% of PBW larvae in the field and they express at a dose 25-fold higher than that needed to kill nearly all the susceptible PBW. Cry1Ac (Event 3006-21-23) is responsible for essentially all the mortality because the Cry1F protein provides virtually no control. Therefore, it is highly likely that resistance to MXB-13 in PBW will be functionally recessive, and thus resistance will evolve only very slowly in the presence of a structured refuge. If PBW that are 25X resistant to Cry1F/Cry1Ac (such as a heterozygote) feed on Cry1F/Cry1Ac cotton (MXB-13), they will be unlikely to complete development. Tabashnik et al (2002) reported that the F1 hybrid of a 3000-fold resistant strain of PBW to the Cry1Ac protein in MVPII® [Mycogen Corp., Bt microbial formulation producing Cry1Ac], initially collected from the field, and a susceptible strain were only 5-fold resistant. These results coupled to the results from the Dow AgroSciences study suggest that if there were resistant insects, that presumably heterozygotes would be killed (functionally recessive) on MXB-13.

C) CBW

Efficacy trials indicated that there was significant survival of cotton bollworm on MXB-13; therefore, Dow AgroSciences designed a study to more accurately quantify the dose.

Results of the two field studies conducted in North Carolina (naturally-infested) and Mississippi (artificially-infested) indicate that cotton bollworm control (large larvae averaged across both locations) by Cry1Ac (alone, MXB-7, and in the pyramid, MXB-13) is very high (~ 93% and ~94%, respectively), while control by Cry1F (MXB-9) is less effective, only 67% of the control. Based on these studies, MXB-13 expressing Cry1Ac and Cry1F insecticidal proteins does not meet the definition of a high dose as one that is sufficient to kill 99.9% of the insects in the field; however, cotton bollworm control was still high, approximately 88% in the Mississippi study and 96% in the North Carolina study. These mortality levels are higher than those for currently registered single protein *Bt* cotton products.

EPA agrees with Dow AgroSciences' assessment that because a high dose of the individual toxins is lacking for CBW, it is less likely that the resistance will be functionally recessive. The risk of CBW resistance is potentially higher than that of TBW. The Dow AgroSciences spatially-explicit, stochastic CBW resistance model is discussed in more detail later in this review.

b. Cross-Resistance Potential

Cross-resistance occurs when a pest becomes resistant to one *Bt* protein that then allows the pest to resist other, separate *Bt* proteins. Cross-resistance poses a risk to pyramided strategies, in which multiple proteins targeting the same pests are deployed simultaneously in the same hybrid. To date, the development of cross-resistance has not been shown in insect pests exposed in the

field to *Bt* crops producing different *Bt* proteins.

After review of the binding studies, EPA agrees that Cry1F and Cry1Ac share some receptors in TBW and CBW, but cross-resistance is expected to be incomplete. In TBW, Cry1F binds to Receptor A, but Cry1Ac binds to both Receptor A and C. In CBW, Cry1F binds to at least four receptors and Cry1Ac binds to at least four receptors, two of which are shared by both Cry1F and Cry1Ac. Thus, a single mutation in a gene that codes for a receptor that binds both Cry1Ac and Cry1F will not prevent all binding of either one; and thus will not allow high survival of an insect bearing even two copies of it, on MXB-13 plants.

Discussions of cross-resistance are complicated due to the fact that the exact nature and genetics of *Bt* resistance are not fully understood. Resistance may vary substantially from pest to pest, adding to the unpredictability of the system. In general, it is possible for resistance to *Bt* proteins to occur through several different mechanisms, some of which may result in cross-resistance to other proteins. As noted earlier, for *Bt* PIPs, two modes of resistance have been seen - detoxification in the midgut lumen by proteases that cleave the PIP and alteration of receptors that prevents binding (Ferré and Van Rie, 2002). Of these, the latter is by far more common. Receptor site insensitivity is likely to have less fitness and is more likely to be mediated by single gene mutations and thus expected to be the faster to evolve. Other mechanisms that may lead to resistance (and ultimately cross-resistance) include protease inhibition, metabolic adaptations, gut recovery, and behavioral adaptations (Heckel 1994, Tabashnik 1994).

The complexity of cross-resistance within a single species or different species is demonstrated by a wealth of experimental evidence. For example, cross-resistance in TBW follows a variable pattern for a closely related group of proteins (Cry1A toxins). An example of a possible shared binding site resulting in cross-resistance was observed with TBW. Gould et al. (1995) selected a TBW strain (YHD2) for a high level of resistance to Cry1Ac (approximately 2000-fold). The YHD2 laboratory-selected strain was found to be cross-resistant to Cry1Aa, Cry1Ab, and Cry1F and showed limited cross-resistance to Cry1B, Cry1C, and Cry2A. Genetic experiments revealed that resistance in the YHD2 strain is partially recessive and is controlled mostly by a single locus or a set of tightly linked loci (Heckel et al. 1997). These results differ from Gould et al. (1992) using a more moderately-resistant laboratory strain of TBW (<50-fold) which showed some broad-spectrum resistance to Cry1Aa, Cry1Ab, Cry1B, Cry1C, and Cry2A. The resistance levels in this TBW strain were low, and subsequent work showed that resistance was inherited as a nearly additive trait (Heckel et al. 1997). Work by Jurat-Fuentes and Adang (2001) indicates that resistance in the YHD2 strain is directed against the homologous domain II loop. Results suggest that it will be difficult to predict what cross-resistance patterns are likely to be in the field because evolutionary responses will depend on the initial frequencies of each resistance allele, the genetic dominance of the alleles, and the mechanism(s) of resistance.

c. Refuge Strategies

The importance of a refuge in insect resistance management is discussed in detail in the section on “Resistance Management Models” below.

d. Adult Effects

Adult effects due to feeding on WideStrike® tissue are not expected to have any impact on the selection intensity (i.e., there are no detectable levels of Cry1F or Cry1Ac proteins in the nectar) and thus, will not decrease the value of the refuge.

e. Technology Adoption Rates and Alternative Controls

The proportion of cotton planted to *Bt* cotton varieties that contain Cry1Ac or both Cry1F and Cry1Ac will affect how much of the pest population is exposed to the PIPs, and thus the level of selection pressure for adaptation. The impact of adoption rates on the evolution of insect resistance will be discussed in more detail in the section on "Resistance Management Models" below.

4. Biological Factors

There are a number of biological factors that impact the evolution of insect resistance: adult movement, larval movement, alternate hosts, population dynamics, and metapopulation dynamics. These will be briefly discussed below.

a. Adult Movement

Adult dispersal among patches (i.e., various hosts) before mating enables the SS genotypes produced by the refuge to mate with RS or RR individuals that may be produced by the *Bt* cotton. The production of RS individuals rather than RR individuals is important in situations where the *Bt* crop produces a high dose against the pest, thus minimizing the fitness differential between individuals carrying the R alleles and those not carrying such alleles. Post-mating dispersal determines where the eggs are laid. The extent of post-mating dispersal can prevent localized foci of elevated R-allele frequency from forming as the R-alleles are diluted across the landscape by S-alleles. That is, post-mating dispersal will ensure the spread of the R-alleles across the region.

1) TBW. Adult dispersal is regarded as moderate (Fitt 1989). That is, there is considerable short range movement between fields and within fields, but longer range dispersal is more limited. Dispersal is more extensive in the spring when adults are looking for suitable non-crop hosts before cotton is available (Peck et al., 1999).

2) PBW. Adult dispersal in PBW is limited in distance, with most insects not dispersing more than ½ mile, although some long-distance dispersal has been observed (Tabashnik et al., 1999). Carrière et al. (2001a) estimated dispersal distances of PBW by tracking movement of males and females from isolated non- *Bt* cotton refuges (source) in surrounding *Bt* cotton (sink). Because *Bt* cotton acts as a deadly sink, moths flying in *Bt* cotton at the end of the growing season (September-November) must originate from refuges. Their results showed that dispersal of females from non- *Bt* cotton to *Bt* cotton was dramatically reduced at only 0.83 km (½ mile) from

the border of the refuge. This work confirmed the earlier results regarding adult dispersal found by Tabashnik et al. (1999).

3) CBW. CBW dispersal is driven by host plant attractiveness. CBW has many wild and crop hosts, and in all cases, is attracted most to the flowering and fruiting structures. CBW adult populations move considerably in areas and at times when there is diversity in host plant phenology, e.g., corn maturation, cotton flowering. Adult movement is less when crops and crop phenology are more uniform such as an area dominated solely by flowering cotton. CBW also exhibits long range migration (Fitt 1989). It is migration that allows CBW to colonize areas of the Corn Belt where it cannot overwinter. A consequence of migration is that the selection pressure for adaptation in one region may not have much effect on the local population's rate of adaptation, as the population mixes significantly with populations in other regions. That is, the range of host plants available in one geographic area may only represent a subset of all the host plants that the local population utilized.

EPA agrees with the Dow AgroSciences' assessment of the adult movement for TBW, PBW, and CBW and the resultant impact on selection pressure.

b. Larval Movement

EPA agrees with the Dow AgroSciences' assessment of larval movement for TBW, PBW, and CBW and the resultant impact on selection pressure. Larval movement can negate the value of the high dose, if it allows partially-adapted heterozygotes to survive better than fully susceptible insects. This situation can arise if the RS insects become established on non- *Bt* plants and then move to *Bt* plants or if the RS insects feed on *Bt* plants and then move to non- *Bt* plants to better survive intoxication than SS insects. Larval movements impact a moderate or low dose situation much less because there is already a considerable fitness difference between RS and SS larvae.

TBW. Larval movement has been observed in TBW. It is not known whether heterozygous insects would experience better subsequent survival than would homozygous susceptible TBW. In any case, to minimize the amount of movement between *Bt* and non- *Bt* plants (or vice-versa) then mixed plantings of *Bt* and non-*Bt* plants should be avoided.

PBW. The larval stage of PBW is spent entirely within a single boll. Boll to boll or plant to plant movement is minimal. Therefore, mixed plantings of *Bt* and non- *Bt* plants would not affect the effectiveness of the high dose. Because of limited PBW larval movement, EPA has allowed narrow in-field strips, at least one row non-*Bt* cotton, for every six to ten rows of *Bt* cotton in the same field, for other cotton PIPs that are currently registered.

CBW. CBW, like TBW, have mobile larvae. However, in the absence of a high dose, the consequence of such larval movement on the population rate of adaption is relatively small, since heterozygote survival is already relatively high compared to SS larvae.

c. Alternate Hosts

Utilization by insect populations of hosts other than *Bt* cotton reduces the selection pressure exerted by the host crop. This contribution to the refuge is applicable at all dose levels. Dow AgroSciencesused the HOSTS database (a database of the host plants of the world's Lepidoptera at http://www.nhm.ac.uk/entomology/hostplants/) as a means of illustrating the potential hosts of TBW, PBW, and CBW.

The utilization and effectiveness of alternate hosts has not been sufficient to prove that non-cotton hosts are effective refuges for TBW, PBW, and CBW. For TBW, alternate hosts do exist, but the ability of alternate (non-cotton) hosts to support complete insect development during the summer is unclear. For PBW, alternate hosts are expected to have no bearing on resistance evolution because they are so limited. Alternate hosts are likely to have the biggest impact on CBW resistance management because of the sheer number of possible hosts and the fact that WideStrike® does not express a high dose of either Cry1Ac or Cry1F. Therefore, the impact of alternate hosts are of greater importance for non-high dose scenarios, due to possible RS survivors (i.e., the need for susceptible SS immigrants from other sources is greater).

Data indicate that CBW are capable of long-distance dispersal and host plants outside the immediate cotton-growing area may act as important sources of non-selected populations potentially diluting resistance. These hosts may lower the metapopulation-wide selection pressure for adaptation, and contribute non-selected insects to the local populations. Yet, empirical evidence is lacking. Both the 1998 and 2000 FIFRA SAP Subpanels concluded that there was very little data to support inclusion of alternate hosts as effective refugia (refuge used here in a broad context, supplying SS moths to dilute resistance). The 1998 SAP Subpanel stated that, "until it is shown that non-cotton hosts produce enough susceptible moths to significantly delay the evolution of resistance in CBW populations exposed to moderate *Bt* doses, non- *Bt* cotton acreage must be considered the primary source of susceptible CBW moths " (SAP 1998). Subsequently, the 2000 SAP Subpanel stated with regard to soybean as a possible refuge that "if there were better empirical data on soybeans, a more realistic model could be developed that accounted for the true year to year variation in the utility of soybean as a refuge" (SAP 2001).

Gould et al. (2002) used stable carbon isotope analysis to assess alternate host use by CBW. They found that non-*Bt* corn in Mexico and the U.S. Corn Belt appears to serve as an important alternate host (non-structured refuge) for CBW. Late-season CBW moths captured in Louisiana and Texas are migrants whose larvae developed on corn in more northern locations. These findings counter the prevailing hypothesis that the majority of late-season moths are produced from larvae feeding on cotton, soybean, and other C_3 plants (most dicotyledon plants using the C3 pathway for photosynthesis). The authors conclude that the non- *Bt* corn refuge is probably more critical to CBW resistance management than the relatively small non- *Bt* cotton structure refuge, and this non- *Bt* corn refuge should be maintained.

Work by Gore et al. (2003) examined the temporal and spatial occurrence of CBW on crop hosts including conventional cotton, soybean, grain sorghum, and field corn in the Mississippi Delta. Stable carbon analyses similar to Gould et al. (2002) were performed. Results indicate that field

corn and grain sorghum provide a good source of susceptible moths during the early season. Grain sorghum may provide sufficient numbers of susceptible CBW for resistance management during some years. However, soybeans do not appear to produce sufficient numbers of CBW for resistance management. Carbon isotope analysis of moths indicated that a significant percentage of the moth population throughout the season developed on host plants other than cotton. The percentage of moths that developed on C_4 plants (e.g., field corn and grain sorghum using the C_4 photosynthesis pathway) never dropped below 25% and for most of the season was greater than 80%. However, the origin of these moths is not clear and more research is needed to investigate the role of wild hosts and long-range migration on the population dynamics of CBW.

While alternate hosts should be considered when attempting to understand pest adaptation and resistance management, empirical evidence regarding their utilization and effective contribution to the production of SS moths to dilute resistance is not known. Dow AgroSciences makes certain assumptions regarding alternate hosts in its CBW modeling efforts discussed later in this review.

Based on the evidence provided to the Agency, until such time as there is sufficient empirical data that demonstrate that alternate hosts are producing insects in sufficient quantity, temporal synchrony, fitness, and proximity to the resistant insects that would be emerging from *Bt* cotton fields, or that susceptible insects from hosts from a longer distance were lowering selection pressure for adaptation (i.e., immigrating metapopulations), then only non-*Bt* cotton can be used as a structure refuge. This uncertainty prompted EPA to require that other *Bt* cotton products (Bollgard and Bollgard II) provide the Agency with additional IRM data to characterize the impact of alternate hosts and supplemental insecticide treatments on refuge effectiveness for CBW, and north-south movement of CBW (EPA 2001, 2003). These same data requirements should also apply to WideStrike® cotton. These data would confirm the Dow AgroSciences CBW modeling predictions and support the theory that external natural refugia in addition to structured refuge reduce the likelihood of CBW adaptation.

d. Population Dynamics

Population dynamics in space and time determine the relative sizes of populations infesting *Bt* and non- *Bt* crops, and thus affect the population-level selection pressure for adaptation. The importance of population dynamics on insect resistance evolution and resistance management is considered in the Dow AgroSciences' product durability plan for WideStrike®. The impact of population dynamics on resistance evolution is simulated in the Dow AgroSciences' CBW model and in the TBW models and will be discussed under the section on "Resistance Management Models."

e. Metapopulation Dynamics

Insect metapopulations are more-or-less subdivided into local populations that are linked by dispersal. Host plants across the geographic range of the metapopulation produce insects that through dispersal contribute to local populations. These non-*Bt* hosts lower the metapopulation-

wide selection pressure for adaptation and contribute unselected insects to the local population. The importance of metapopulation dynamics is considered by Dow AgroSciences in its CBW model and will be discussed under the section on "Resistance Management Models."

5. Genetic Factors

Along with the various operational and biological factors discussed above, Dow AgroSciences discusses the impact of a number of genetic factors that impact the evolution of insect resistance: genetic dominance, initial R frequency, cross-resistance amongst *Bt* Insecticidal Crystalline Proteins (PIPs), and cross-resistance with other control mechanisms. These are briefly discussed below.

a. Genetic Dominance

EPA agrees with the Dow AgroSciences discussion of genetic dominance of an R-allele and its importance. The genetic dominance of an R-allele determines the potential functional dominance. If the R-allele is genetically completely recessive (i.e., dose-response of RS = dose-response of SS), then it will also be functionally recessive irrespective of the dose of the PIP (i.e., RS survival = SS survival). If an R-allele is completely genetically dominant (i.e., dose-response of RS = dose-response of RR), then it will also be functionally dominant irrespective of the dose of the PIP (RS survival = RR survival). If the RR has low survival on the *Bt* plant, then the selection pressure favoring the R allele will be weak. If the R-allele is nearly completely recessive (i.e., dose-response of RS is close but slightly higher than for SS), then the functional dominance of resistance is likely to be low, even at non-high doses. That is, heterozygote survival will not be much higher than SS survival on the plant. If the R-allele is additive (i.e., dose response of RS is halfway between those for SS and RR), then the functional dominance is highly sensitive to dose if it is not high (i.e., a small change in dose can have a large effect on the difference between RS and SS survival).

For *Bt* PIPs and resistance mediated through a receptor binding change, the expectation is that the R-alleles will be genetically recessive to incompletely recessive, as resistance is mediated through a loss of function. Resistance that is mediated through a gain of function, for example if a digestive enzyme is novel or expressed at much higher levels, then the expectation is that the R-allele will be incompletely dominant. In their review of the binding site modification data related to *Bt* resistance, Ferré and Van Rie (2002) found that resistance was due to a recessive or partially recessive mutation in a major autosomal gene.

b. *Initial* R Frequency

EPA agrees with the Dow AgroSciences explanation that *Bt*-resistance allele(s) frequency will likely be low in the population for TBW, CBW, and PBW. As noted above, Gould et al. (1997) estimated the field frequency of Cry1Ac major resistance alleles in TBW as 1.5×10^{-3} (4.1×10^{-3} the upper bound of the 95% confidence interval) . Burd et al. (2001) estimated that the frequency of resistance to Cry1Ac was 4.3×10^{-4} indicating that resistance was rare, although inheritance of

resistance was incompletely dominant. Reports of resistance allele frequency for PBW have been variable as noted above, from undetectable to 0.16 (Tabashnik et al. 2000). Subsequent work to Tabashnik et al. (2000) explains that the lack of field failure due to PBW resistance in the population is due to fitness costs associated with resistance (reduced overwintering survival and reduced survival on non- *Bt* cotton plants) and maternal effects (Carrière et al. 2001b and c). Resistance monitoring work in 2001 and 2002 in Arizona has shown that resistant PBW were detected in the field (0.172% survival at the 10 μg/ml), but at much lower frequencies than in 1997 (R-allele frequency = 0.16) and efficacy in the field remained unchanged (Dennehy et al. 2003).

c. Cross-Resistance Amongst Bt PIPs

See earlier discussion on cross-resistance. EPA recognizes the potential for Cry1Ac and Cry1F to confer cross-resistance in TBW and CBW because Cry1F and Cry1Ac share some binding sites. However, the cross-resistance is not expected to be complete because of the number of binding sites involved. PBW is not susceptible to Cry1F and thus cross-resistance to Cry1F is not an issue.

d. Cross-Resistance With Other Control Mechanisms

EPA agrees with the Dow AgroSciences' assessment. Cross-resistance between *Bt* and other chemical insecticide classes is not expected based on differences in mode of action. Chemical insecticides have been used to control TBW, CBW, and PBW. These include the following classes: pyrethroids, carbamates, spinosyns, and organophosates, as well as others. Cross-resistance between Bt and these other classes has never been documented and is not expected based on the mode-of-action.

6. Resistance Management Models

Computer models can provide an objective synthesis of the complex interaction among the operational, biological, and genetic factors discussed above and provide a scientific basis for understanding the overall impacts of product durability strategies on the rate of pest adaptation. Dow AgroSciences provides an analysis of TBW and CBW resistance management models as they pertain to WideStrike® cotton.

a. TBW

EPA reviewed the Peck et al. (1999) stochastic, spatially-explicit, simulation model that examined factors that may influence the regional development of TBW resistance to Cry1Ac (see EPA 2001). A brief summary is provided here. Using this model, they found that the spatial scale and the temporal pattern of refuges can have a strong effect on the development of TBW resistance to *Bt* cotton. Specifically, the time to resistance was significantly longer (49 years) in regions where the same fields were used as a refuge from year to year and adult movement among fields is limited. In regions where the refuge fields are changed randomly from year to year, the

region develops resistance more quickly (17 years). Peck et al. (1999) concluded that it would only take a minority of growers who do not employ refuges properly to start a regional resistance problem. These authors found that 20% (sprayed) refuges did delay resistance. They noted that a delay in larval development on *Bt* plants can alter the rate of resistance development to increase or decrease the rate of resistance development. They commented that designing controls to limit the overwintering potential of the last generation may be effective in slowing resistance. Exploring the interaction among parameters is very difficult with this complex model, but this type of model is useful to examine a number of challenges to managing resistance in *Bt* cotton (e.g., how the refuge is managed year to year) and the scale (regional level) of management of resistance. Neither the spatial scale nor temporal pattern of placement of refuges has been investigated in the field.

EPA agrees with Dow's analysis that the rate of adaptation of TBW to WideStrike® will always be slower than that predicted in the Peck et al. model. WideStrike® (MXB-13) expresses a high dose of Cry1Ac/Cry1F against TBW. Cry1F is expressed at nearly a high dose in cotton line MXB-9 and Cry1Ac is expressed at a high dose in cotton line MXB-7 (Blanco et al. 2002; MRID#45808417). Thus the survival parameter in the model, 0.01, should be lowered, by at least 10-fold. The resistance allele frequency used in the Peck et al. model was 0.03, a value that is much higher than is estimated. Population adaptation will thus be slower than predicted by the Peck et al. model. Finally, WideStrike® expresses two PIPs, rather than a single PIP, as modeled by Peck et al. Modeling predicts that the durability of a two-gene pyramid will always be greater than a single-gene PIP (Roush 1998, Caprio 1998, Zhao et al. 2003). Zhao et al. (2003) demonstrated that *Bt* broccoli plants expressing two *Bt* toxins will delay diamondback moth (*Plutella xylostella*) resistance more when compared to single toxins used sequentially or in a mosaic.

b. CBW

EPA has used several CBW models to understand adaptation to *Bt* cotton expressing Cry1Ac in different environments (EPA 2001; Matten and Reynolds, 2003). Each of these models indicates that the risks for CBW adaptation are somewhat higher than for TBW under cotton-only and cotton plus corn scenarios.

One modeling effort in particular, Storer et al. (2003), was the basis for the Dow AgroSciences' CBW model detailed in this submission (MRID# 45808415). The Storer et al. model was adapted from Peck et al. (1999). The Storer et al. (2003) spatial, stochastic computer model was developed to simulate the evolution of resistance in *H. zea* (CEW/CBW) to *Bt* cotton in an agroecosystem that includes both *Bt* corn and *Bt* cotton, such as eastern North Carolina. Using this model, the authors found that selection for resistance is more intense in *Bt* cotton fields than in *Bt* corn fields. For example, the R-allele frequency if 75% of cotton is *Bt* and 25% of corn is *Bt* increased more rapidly than if 25% of cotton is *Bt* and 75% of corn is *Bt*. Storer et al. concluded that the greater importance of *Bt* cotton with regard to resistance development was due to spraying of non-*Bt* cotton fields when they reached economic threshold levels which reduced the effective refuge size. The spatial distribution of transgenic and non-transgenic plantings can

affect the region-wide evolution of resistance and, especially when the on-farm refuge size is small, the resistance levels in sub-populations. They concluded that farm-level refuge requirements are important even for a highly mobile pest such as *H. zea*. Once established, *H. zea* resistance could spread to farms in regions that do not use *Bt*.

The Dow AgroSciences' CBW model was extended from the Storer et al. (2003) model to include two additional transgenic cotton proteins (for a total of three: Cry1Ac, Cry1F, Cry2Ab) and three protein receptors. The model simulates two agroecosystems, North Carolina and the Mississippi Delta, both consist of the CBW crop hosts soybean, maize, and cotton in varying amounts. The model simulates 15 years of deployment of *Bt* maize and *Bt* cotton. Assumptions are detailed earlier in the review. Sensitivity analyses indicated the following input parameters were most critical: amount of insect product from soybean fields, soybean flowering dates, immigration of non-selected populations, initial R-allele frequency, and fitness costs of R-alleles. The Dow AgroSciencesCBW model represents a worst case scenario because it assumes that there are only three protein binding receptors, fewer than observed in binding studies. In the absence of field resistance to either Cry1Ac or Cry1F, it is impossible to predict with any accuracy how insects carrying one or more R-alleles will survive on WideStrike®. Based on the conservative nature of the assumptions in the Dow AgroSciences' CBW model, there is a low probability that there will be a significant change in population fitness of CBW on WideStrike® in a 15-year time horizon even without a high dose and incomplete cross-resistance (20 to 60% maximum shared binding). Resistance evolves more slowly under conditions of incomplete cross-resistance than when there is no cross-resistance ($x = 0$) or when there is complete cross-resistance ($x = 1$). Binding data indicate that these intermediate levels are appropriate for Cry1F and Cry1Ac. In the North Carolina agroecosystem, the 15-year population fitness increased somewhat with market share of WideStrike®. While in the Delta agroecosystem, the 15-year population fitness decreases with market share of WideStrike® due to the influence of the immigrant population. Refuge size had no significant impact on CBW population fitness on WideStrike® after 15 years in either the North Carolina agroecosystem or the Delta agroecosystem.

Previous modeling efforts by Roush (1998), Caprio (1998), and Zhao et al. (2003), have predicted that the durability of a two-gene pyramid will always be greater than a single-gene protein. In addition, a bioeconomic model by Livingston et al. (2002) predicts that the addition of a second protein to an existing single protein variety decreases the risk of resistance to the initial protein, while increasing the risk of resistance to the new protein. Dow AgroSciences' CBW modeling efforts confirm the same conclusions as derived from the previous modeling efforts. Dow AgroSciences' efforts indicate that WideStrike® cotton will have predicted advantages over a single protein product even when there is some cross-resistance and when there is somewhat less than a high dose for either protein.

1) Model Input

The Dow AgroSciences CBW model is spatially-explicit and stochastic and was adapted from the CBW model originally described in Storer et al. (2003). The Dow AgroSciences' CBW model was extended to include two additional transgenic cotton PIPs (for a total of three: Cry1Ac,

Cry1F, Cry2Ab) and three protein receptors. The model simulates an agroecosystem consisting of the CBW crop hosts soybean, maize, and cotton. The model simulates 15 years of deployment of *Bt* maize and *Bt* cotton. The insects primarily utilize maize for the first two generations and cotton for the second two generations each year. Weed hosts are also utilized in the first and last generations, soybean in the second and third. For these model runs, two agroecosystems are simulated. The first agroecosystem approximates the crop mix in North Carolina: 50% soybean, 25% maize, and 25% cotton. The soybean and cotton acres are randomly mixed. The second agroecosystem represents the crop mix in the Mississippi Delta: 62% soybean, 8% maize, and 30% cotton. In the Delta, the soybean and cotton acres are not randomly mixed. Because of the way the region's edges are modeled, the insects at the edge are "bounced back" into the region. This corresponds to a large area dominated by cotton surrounded by soybean acreage. The maize is scattered randomly throughout the region. In both agroecosystems, crops are assigned to fields randomly each season. Annual crop phenology, from pre-flowering, through flowering and maturity to harvest follows the statewide averages for crop progress for North Carolina and Mississippi, respectively (USDA-NASS website).

In the North Carolina system, 50% of the maize is planted to hybrids expressing Cry1Ab, which for these purposes is assumed to share binding (complete cross-resistance) with Cry1Ac, while in the Delta system, none of the maize expresses Cry1Ab. Cotton fields can be sprayed if populations reach threshold (150,000 eggs per ha or 16000 larvae per ha on non-*Bt*; 1,500,000 eggs per ha on *Bt* cotton). Similarly, soybean can be sprayed if the population reaches 46,000 larvae per ha threshold during flowering or early pod set.

For the Mississippi Delta agroecosystem, early season immigration occurs before the local population emerges from diapause based on published research (e.g., Fitt 1989). This means that the local rate of adaptation depends in part on the resistance frequency of the immigrating population, which in turn depends on the selection history of the source population. Immigrating moths are likely coming from southern Texas, Mexico, and the Caribbean; areas in which selection pressure is low under current deployment levels of *Bt* corn and cotton.

2) PIPs, PIP Binding and Insect Fitness

The *Bt* proteins in the model are Cry1F, Cry1Ac, and Cry2Ab. What is understood of the binding of Cry1F and Cry1Ac in TBW and CBW has been previously discussed. It is assumed that the binding sites of Cry2Ab proteins are not shared with those for Cry1 proteins. Dow AgroScienceshas simplified the binding map for the purposes of the CBW model. The simplification is highly conservative, as it only requires changes at three receptors for an insect to be resistant to all three proteins; whereas, changes in upward of seven receptors may be needed for complete cross-resistance.

The amount of cotton that is planted to four different *Bt* cotton types is varied in the model. The four types are: a) varieties expressing Cry1Ac alone (e.g., Bollgard); b) varieties expressing Cry1Ac plus Cry2Ab with no cross-resistance (e.g., Bollgard II); c) varieties expressing Cry1F plus Cry1Ac (MXB-13); and d) non- *Bt* varieties. Type a) (Cry1Ac alone) is assumed to kill

80% of the susceptible CBW based on published field data (e.g., Lambert et al. 1997). Type b) is assumed to kill 96% of the susceptible CBW, whereby the second protein (Cry2Ab) kills 80% of the survivors of Cry1Ac. Mortality of type c), cotton line MXB-13, depends on the mortality inflicted by each PIP alone and on the degree of shared binding. For the purposes of the model, the Cry1F line (MXB-9) is assumed to inflict 67% mortality, the Cry1Ac line (MXB-7) is assumed to inflict 99% mortality, and Cry1F/Cry1Ac line (MXB-13) is assumed to inflict 97% mortality (Storer and Blanco 2002, MRID# 45808418). The mortality of the Cry1F/Cry1Ac pyramid (MXB-13) is determined by the degree of shared binding. Results from Sheets and Storer (2001) indicate that around 60% of Cry1Ac binding is to molecules that also bind Cry1F (receptor A), while the remaining 40% binds to receptors that do not bind Cry1F (receptor B). If there was no shared binding, *Bt* proteins in MXB-13 would kill 99% of the susceptibles; if there was complete overlap (complete cross-resistance), the combination would kill 97.1% of the susceptibles. Simulations were also run using field data from Mississippi (Storer and Blanco 2002; MRID#45808418) as inputs, but the model was not sensitive to input values for mortality.

Understanding the mortality of insects carrying one or more R-alleles is important to understanding the durability of the product. Mortality depends on the functional dominance of resistance on each *Bt* cotton type, and the value of ***x*.** Sheets and Storer (2001) indicated that around 60% of Cry1Ac binding is to molecules that also bind Cry1F (receptor A), while the remaining 40% binds to receptors that do not bind Cry1F (receptor B).

The R-alleles are assumed to be functionally additive (i.e., functional dominance = 0.5) on *Bt* cotton due to the lack of a high dose against CBW. There are two loci at which R-alleles can lead to adaptation to cotton line MXB-13; one for receptor A and one for receptor B. A mutation at the locus for receptor B will not affect Cry1F and Cry2Ab binding, but will affect Cry1Ac binding. A mutation at the locus for receptor A will not affect Cry2Ab binding, but will affect Cry1Ac and Cry1F binding. A mutation at the locus for receptor C will not affect Cry1Ac and Cry1F binding, but will affect Cry2Ab binding. For complete resistance to Cry1F, only the locus for receptor A must be homozygous for R-alleles. For complete resistance to Cry1Ac, both loci - receptors A and B, must be homozygous for R-alleles. On Cry1F cotton, the survival only depends on the genotype for receptor A. On Cry1Ac cotton, survival depends on the genotype for receptors A and B. On MXB-13 cotton expressing both Cry1F and Cry1Ac, the fitness is calculated as the product of the survival of binding at each receptor since some of the Cry1Ac activity overlaps with the Cry1F activity. Dow AgroSciences has provided tables for fitness of all 27 insect genotypes on each cotton type at three different levels of shared binding.

It is assumed in this study that all specific binding of Cry1Ac is functional. That is, all binding events are followed by incorporation of the protein into the gut membrane which results in a functional pore leading to cell lysis. Non-functional binding of the protein to a receptor with formation of a functional pore is also possible, but this would make the analysis considerably more complicated. Adaptation to *Bt* protein PIPs is assumed to be caused by mutations to the midgut receptors that were identified in the ligand-binding study (i.e., Sheets and Storer 2001) and that each receptor requires a different mutation. Therefore, to be completely adapted to both Cry1Ac and Cry1F, an insect would have to be homozygous to two receptor mutations.

Furthermore, insects heterozygous for an adaptation allele will have a fitness exactly half-way between that of homozygous susceptible insects and that of homozygous resistant insects (i.e., dominance of the adaptation trait is additive). The Dow AgroSciences' model represents a worst case scenario because it assumes that there are only three protein binding receptors, fewer than observed in binding studies.

Initial Gene Frequency. As discussed above, it is expected that initial frequency of the R-alleles will be rare. In the model, the initial (unmutated) R-allele frequency for each receptor was assumed to be 0.001. Assuming Hardy-Weinberg equilibrium before selection (no mutation, no fitness costs), 1 in 1,000,000 individuals will be homozygous for the mutated form of one of the receptors. Similarly, 4 in 1,000,000 ($2*0.001^2*(1-0.001^2)$) will be heterozygous for the mutated form of two receptors.

3) Model Output: **Model Runs and Results**

Model output is expressed as population fitness on *Bt* cotton after 15 years of deployment. As with other models, this should not be regarded as predictive as there are many uncertain processes (e.g., weather) that are not included in the model. The model output is used for comparative purposes to examine the effects of certain parameters and scenarios on pest adaptation and thus, to examine the effectiveness of different product durability programs.

a) Level of Shared Binding

Shared binding sites by Cry1F and Cry1Ac leads to the expectation of some level of cross resistance, i.e., individuals carrying R-alleles at the locus for the shared binding site will show enhanced survival against both Cry1Ac and Cry1F. However, in the absence of resistance in CBW, it is unclear how much each binding site contributes to mortality. A sensitivity analysis was conducted.

Dow AgroSciences simulated the effect of the level of shared binding on the durability of the product, as measured by the change in mean population fitness on MXB-13 (WideStrike®) after 15 years of deployment alongside Bollgard. These runs were conducted in the North Carolina scenario, with 40% of cotton planted to WideStrike®, 40% to Bollgard and 20% as refuge non-*Bt* cotton. As anticipated, when there is completely shared binding ($x = 1$) of Cry1F and Cry1Ac, adaptation to WideStrike® occurs most quickly since only one locus needs to be resistant. At completely independent binding ($x = 0$), adaptation occurs significantly more slowly since two R-alleles at two loci are required. In this situation, resistance to Cry1Ac is selected for on Bollgard cotton, WideStrike® cotton and on *Bt* corn, whereas resistance to Cry1F is selected on only WideStrike®. Since the vast majority of insects that are heterozygous for resistance to Cry1F are still fully susceptible to Cry1Ac (while Cry1Ac resistance is rare) there is little survival of these insects and the Cry1F R-allele does not increase in frequency.

At intermediate levels of shared binding, selection at both loci occurs on all *Bt* cotton and *Bt* corn. Selection pressure exerted by Cry1Ac is greater than that exerted by Cry1F because

Cry1Ac is present in all *Bt* cotton and all *Bt* corn (both Cry1Ab and Cry1F are expressed in different hybrids). Resistance to Cry1Ac requires more than one receptor change so it evolves more slowly than when *x* was set to 1 or 0. At these intermediate levels, adaptation to both Cry1Ac and Cry1F occurs more slowly. As noted earlier, binding data indicate that these intermediate levels are appropriate for this pair of molecules. For the remaining runs of the model, the default value for *x* is 0.6.

Additional model simulations were run using the dose data from Mississippi (Storer and Blanco, 2002; MRID# 45808418) as input parameters for mortality on WideStrike® PIPs. In this field study, mortality of the Cry1F-expressing parent line (MXB-9) was around 70%, while mortality on the Cry1Ac-expressing parent line (MXB-7) was about 88%, and the mortality on the pyramid (MXB-13) was around 92%. Results of these runs indicated a similar change in population fitness over 15 years to that for the default mortality parameters (i.e., MXB-9 67% mortality, MXB-7 99% mortality, and the pyramid 97% mortality). These results indicate that the model is not very sensitive to the actual dose of the two *Bt*.proteins, given that they are not high dose against CBW.

In all ligand-blot binding studies of Cry1 proteins, each protein has been shown to bind to more than one receptor in the target insects. By pyramiding two *Bt*.proteins (e.g., Cry1F + Cry1Ac), the range of receptors involved in resistance is expanded, and the selection pressure for resistance at one receptor is reduced. The binding map used in the model is an oversimplification that assumes that there are no additional receptors involved in toxicity of either protein.

b) Market Share Modeling

It is expected that WideStrike® will share the *Bt* cotton market with Bollgard®, Bollgard® II or both. As discussed above, the complex of PIPs involved in these products reduces the selection pressure for resistance to any one, especially given the complexity of binding receptors.

The impact of WideStrike® market share on fitness of Bollgard® after 15 years was very small. In the North Carolina agroecosystem, the 15-year population fitness increases somewhat with market share of MXB-13. Conversely, in the Mississippi Delta agroecosystem, the 15-year population fitness decreases with market share of WideStrike®. This is due to a decrease in the population surviving each year and a resulting increase in the influence of the immigrant population (which is unselected in the model runs).

Modeling of market share of WideStrike® in competition with Bollgard® II resulted in slower adaptation than market share with Bollgard®, as insects are faced with three different *Bt* proteins. This indicates that as Bollgard® II replaces Bollgard®, the rate of adaptation to WideStrike® will be slowed (that is, lower fitness on WideStrike® after 15 years in the presence of Bollgard® II than after 15 years in the presence of Bollgard®). Correspondingly, a slower rate of adaptation to Bollgard II is anticipated to occur in the presence of WideStrike® than with Bollgard® as the sole PIP within the cotton market.

c) Sensitivity Analysis

A sensitivity analysis was conducted to investigate the effect of many of the parameters of this model. The effects of the following parameters were found to be most important: the amount of insect product from soybean fields, soybean flowering dates, immigration of non-selected populations, initial R-allele frequency, and fitness costs of R-alleles. The parameters with moderate effects were: functional dominance of R-alleles on each crop, dispersal probability, and larval development duration.

In the Delta agroecosystem, additional sensitivity analyses were conducted. These analyses showed that the R-allele frequency of the immigrating population can overwhelm local selection and act as a driver for adaptation since local adaptation occurs very slowly. At the default setting where immigrant population is at the pre-selection R-allele frequency, the size of the immigrant population, even zero, did not affect local adaptation. This means that local resistance evolution in the Delta agroecosystem is very slow due to the binding patterns and alternate hosts. Other parameters with significant effects in the Delta were: the proportion of soybean in the region, survival of the final, fall population on cotton, and the spray threshold for cotton.

c. PBW Modeling

Dow AgroSciences does not include any discussion of PBW resistance models. PBW models for purposes of examining resistance evolution under a variety of mitigating strategies do not currently exist. Carrière et al. (2003) used multiple regression and two different population dynamics models (one deterministic, the other stochastic, similar to Peck et al. 1999, described earlier) to show that high use of *Bt* cotton (threshold = 0.65 or 65% *Bt* cotton) led to regional PBW population declines in Arizona. This is important work because, as the authors note, insecticide sprays have not caused long-term suppression of PBW in Arizona. The authors conclude that long-term regional suppression of PBW may further reduce insecticide use and enhance implementation of the EPA-mandated refuge requirements for *Bt* cotton. In future submissions, it is recommended that Dow AgroSciences include pink bollworm PBW resistance modeling in its product durability analysis to determine the relative expected efficacy of its proposed IRM strategy for PBW.

7. IRM Tools

The above analysis indicates that the IRM tools available for managing product durability (insect resistance management) vary by pest. Key points are summarized based on the analysis above.

The complex of *Bt* proteins involved in WideStrike®, Bollgard®, and Bollgard® II reduces the selection pressure for TBW or CBW resistance to any one *Bt* protein, especially given the complexity of binding receptors. TBW and CBW binding studies involving Cry1F and Cry1Ac indicate that there are at least two, and probably at least six binding sites for these two proteins. For TBW, WideStrike® is at a high dose against TBW, the Cry1Ac-expressing parent line is also at a high dose, and the Cry1F-expressing parent line is highly efficacious. The Peck et al. (1999)

model showed that a high dose of a single PIP with a 20% refuge is a durable plan for TBW. The addition of a second protein (Cry1F + Cry1Ac in MXB-13) makes the 20% refuge even more durable and reduces the refuge size needed for the same level of protection across a 15-year time horizon. For CBW, neither Cry1F nor Cry1Ac is expressed at a high dose in WideStrike®; although Cry1Ac mortality is much higher in WideStrike® than for Bollgard® cotton and Cry1F efficacy is moderate. This pest has numerous alternate hosts and is highly migratory. This reduces the role of local selection pressure at the local population level and increases the role of metapopulation-wide selection pressure. The planting of non-*Bt* cotton refugia in or close to all *Bt* cotton fields contributes to lowering the metapopulation selection pressure.

For PBW, WideStrike® is a high dose for Cry1Ac. Cry1F has no apparent control of PBW based on field efficacy data (Pellow 2002; MRID# 45808407) and high dose data (Storer and Richardson 2003; MRID# 46071901) and thus, WideStrike® effectively expresses a single protein (Cry1Ac) to control PBW. A small structured refuge in combination with the high dose, planted as close as practicable to the *Bt* cotton, would increase the WideStrike® durability. Use of resistance management models would confirm the relative durability of its proposed IRM plan for PBW.

For TBW, the addition of a second protein, makes the 20% refuge even more durable than for a single protein expressed at a high dose and reduces the refuge size (as compared to a single protein, high dose product needed for the same level of protection as predicted by Peck et al. (1999) across the same time horizon. Further refinement of the Peck et al. (1999) model would improve the predictions of time to likely resistance development.

For CBW, neither Cry1F nor Cry1Ac is expressed at a high dose in WideStrike®. Population fitness is lowest with intermediate levels of shared binding of Cry1F and Cry1Ac. Some level of shared binding is expected for Cry1F and Cry1Ac based on binding studies. Sheets and Storer (2001) indicate that 60% of Cry1Ac binds to the Cry1F receptor in CBW. Even in the simplification of binding receptors, an insect would have to be homozygous for two receptor mutations to be completely adapted to both Cry1F and Cry1Ac. Given that the model assumes fewer binding sites than were observed in binding studies, complete adaptation in the field would require at least six or more receptor mutations. Modeling runs also indicate that refuge size is not very important to management of CBW resistance since WideStrike® is a pyramid of two proteins with limited cross-resistance and is inherently durable when coupled with the natural refugia from alternative hosts. More empirical data need to collected to validate the effectiveness of nature refugia from alternate hosts. Thus, a 20% sprayable refuge is likely to be more than adequate for prolonging durability against CBW, and will be most important in areas where there is little immigration (as in North Carolina) or where the productivity of CBW from alternative crop hosts is limited (as in the Mississippi Delta). The 5% unsprayed option alone is not as effective as the 20% sprayed under the assumptions and parameter settings used in the model. However, given that the model is highly conservative, and that there is little change in population fitness after 15 years, this option should also be considered durable.

8. IRM Plan for WideStrike® cotton

Based on all of the data discussed above, Dow AgroSciences proposed the five point IRM Plan for WideStrike® cotton summarized below.

1. 5% external unsprayed refuge option. Five percent of the cotton fields must be planted to non-*Bt* cotton and not be treated with any lepidopteran-control technology. The refuge must be at least 150 ft. wide (preferably 300 ft.) and within ½ mile (preferably adjacent or within 1/4 mile or closer) of the *Bt* cotton.

2. 20% external sprayable refuge option. Twenty percent of the cotton fields must be planted to non- *Bt* cotton and may be treated with lepidopteran-active insecticides (or other control technology) except for microbial *Bt* formulations. The refuge must be within 1 mile (preferably within ½ mile or closer) of the *Bt* cotton fields.

3. 5% embedded refuge option (for TBW and CBW). Five percent of a cotton field (or fields) must be planted with non-*Bt* cotton as a block within a single field, at least 150 ft. wide (preferably 300 ft. wide) or single field blocks within a one mile squared field unit. The refuge may be treated with lepidopteran-active insecticides (or other control technology) only if the entire field or field unit is treated at the same time.

4. Embedded (in-field strip) refuge option for PBW. One single row of a non-*Bt* cotton variety must be planted for every 6 to 10 rows of *Bt* cotton. This can be treated with lepidopteran-active insecticides (or other control technology) only if the entire field is treated at the same time.

5. Community refuge option. Farmers can combine neighboring fields within a one-mile squared field unit that act as a 20% sprayable refuge or the 5% unsprayed refuge. Participants in the community refuge option must have a community refuge coordinator and appropriate documentation is required.

Both EPA and the June 2004 FIFRA SAP agree that the Dow AgroSciences proposal for the above refuge options should be appropriate for insect resistance management to WideStrike® and will afford clarity and consistency of the IRM to growers, consultants, extension entomologists, seed dealers, and others that need to understand and implement it (See page 59 of the June 2004 FIFRA SAP Report).

The Dow AgroSciences' CBW modeling efforts indicate that there will not be a significant change in population fitness of CBW on WideStrike® in a 15-year time horizon even without a high dose and incomplete cross-resistance (20% to 60% maximum shared binding). The June 2004 SAP, however, recommended further examination of the model. Refuge size, whether sprayed or unsprayed, had no significant impact on CBW population fitness on WideStrike® after 15 years. In the Delta the immigrating non-selected population further reduces the local rate of adaptation. The local structured refuge only supplies a small proportion of the non-selected insects in the Delta. For TBW, which exhibits similar patterns in binding studies, against which WideStrike® is a high dose and against which the Cry1Ac component alone is a high dose, durability will be even greater than is predicted for CBW and that which was predicted using the TBW model by Peck et al. (1999). For PBW, WideStrike® expresses a high dose of Cry1Ac.

Although WideStrike® selects for R-alleles at the genes encoding receptors for Cry1Ac, this is balanced by the presence of Cry1F reducing survival of Cry1Ac-resistant insects. The precise population biology in any given area in any given year greatly influences the balance of these competing forces. The same affect applies equally to TBW and CBW. Because the model does not include any Cry1F-only receptors (which are known to exist), it underestimates the mortality of Cry1Ac-resistant individuals on WideStrike® and therefore underestimates the magnitude of the Cry1F effect delaying resistance to Cry1Ac. Just as predicted, evolution of resistance to Cry1Ac is greatly delayed when the number of Cry1Ac binding sites is increased from one to two, so the evolution of resistance to Cry1F is predicted to be similarly delayed when additional Cry1F receptors are included in the model. Under typical cotton production practices, it is expected that the Cry1F in WideStrike® will be durable and will reduce the rate at which Cry1Ac-resistance evolves in TBW and CBW.

It is also important to note that recent labeling schemes encouraged by EPA and the chemical insecticide industry encourage growers to use multiple modes of action in controlling insects in order to reduce the likelihood of insects evolving resistance to any one control agent. Following this principle, use of WideStrike® in an agroecosystem where other chemical control measures are also used reduces the selection pressure for resistance to each measure.

9. Grower Implementation (Education and Compliance)

Dow AgroSciences notes that ensuring growers plant and manage refuges in the required manner is an important element of their product durability plan, especially for managing adaptation to TBW and PBW because of their comparatively limited host range, limited adult dispersal, and the high dose. To that end, Dow AgroSciences will implement a multi-pronged effort to educate growers and measure the level of refuge implementation.

Education and compliance with IRM requirements are critical elements for successful resistance management. Significant non-compliance with IRM among growers may increase the risk of resistance for *Bt* cotton. However, it is not known what level of grower non-compliance will compromise the risk protection of current refuge requirements. *Bt* cotton grower education has been reviewed in EPA's White Paper (EPA 1998) and was emphasized at the EPA/USDA

Workshop on *Bt* cotton IRM held in August 1999 (EPA/USDA 1999). The 2000 SAP Subpanel stressed the importance of grower education and its impact on grower compliance (SAP 2001). Because of the recommendations made by the June 2004 SAP and many stakeholders, EPA is requiring specific grower education and compliance programs for WideStrike®.

10. Baselines, Resistance Monitoring and Mitigation (Remedial Action)

Resistance monitoring. The need for proactive resistance detection and monitoring is critical to the survival of *Bt* PIP technologies. Early detection of significant changes in resistance allele frequency (that will lead to field resistance) is necessary. This will allow IRM plans to be potentially altered prior to field failure.

Dow AgroSciences has described the basic elements of its proposed resistance monitoring program. The proposed program has a route for reporting and investigating suspected cases of resistance and one for confirmed resistance. Dow AgroSciences proposed to collect 15 to 20 populations each of TBW and CBW and perform laboratory bioassays to determine whether there are any changes to the susceptibility of these insects to either Cry1F and/or Cry1Ac. Sampling will be focused in areas of highest adoption. Similarly, 4 to 6 populations of PBW will be collected across Arizona, New Mexico, and California and examined for changes in insect susceptibility. EPA agrees with Dow AgroSciences that the resistance monitoring program should be focused in areas of highest adoption in which selection pressure is expected to be highest. Baseline susceptibility data for WideStrike® collected during 2002 and 2003 are still being analyzed for TBW, CBW, and PBW. Based on the baseline data, a discriminating dose for Cry1F and Cry1Ac will be established.

The currently required resistance detection method for *Bt* resistance is the discriminating dose/diagnostic dose bioassay system that would distinguish between resistant and susceptible phenotypes. However, such tests have been criticized as being too insensitive to be able to provide early detection before resistance develops or can spread very far, especially if the alleles for resistance are rare in the insect population. Discriminating dose bioassays are most useful when resistance is common (homozygous recessive alleles, i.e., field failure levels) or conferred by a dominant allele when the resistance allele frequency is greater than 0.01 (Andow and Alstad, 1998; Andow et al., 1998). It is currently considered as one of the central components of any monitoring plan, but other monitoring methods, such as the F_2 screen and DNA markers, may have value in conjunction with the discriminating concentration assay.

Dow AgroSciences must provide EPA the baseline susceptibility data for Cry1F and Cry1Ac for the 2002 and 2003 growing season, establish diagnostic/discriminating concentrations for tests for resistance to Cry1F and Cry1Ac, and provide a detailed resistance monitoring plan for both Cry1Ac and Cry1F. Dow AgroSciences may choose to coordinate its monitoring efforts for WideStrike® with the current resistance monitoring programs for other *Bt* cotton PIPs.

Remedial action plans. A Remedial Action Plan defines not only suspected and confirmed resistance, but also the key steps and actions needed if and when resistance develops.

Dow AgroSciences needs to prepare specific remedial action plans for WideStrike® to address TBW, CBW, and PBW resistance if it is suspected or actually does occur. While the general elements of the remedial action plans for suspected and confirmed resistance are noted by Dow AgroSciences, these plans need more detail.

Generally, if resistance is confirmed, the farmers involved will treat their *Bt* crop with alternative pest control measures. This might be a chemical pesticide known to be highly effective against the insect or it might mean measures such as crop destruction. In addition, the sale and distribution of the *Bt* crop would be suspended in that area and the surrounding area until it can be determined that insects in that area have regained their susceptibility to the *Bt* proteins. Other registrants with the same (or similar) *Bt* proteins would be notified. There would also need to be increased monitoring to define the remedial action area(s). Other remedial action strategies include increasing refuge size, changing dispersal properties, use of sterile insects, or use of other modes of actions. Geospatial surveys would help define the scale of remedial action and where to intensify monitoring. Because no *Bt* field resistance has yet been found, all of these tactics are untested. The greatest concern with remedial action plans is that they may not work either to eradicate resistance or mitigate it. This concern was noted by the 2000 SAP Subpanel (SAP 2001).

11. On-going Research

EPA recommends that Dow AgroSciences provide the Agency with relevant IRM research applicable to WideStrike® IRM. IRM research is desirable to refine TBW and CBW resistance models and to develop PBW resistance models.

To support alternate hosts as effective refuges of CBW, Dow AgroSciences should supply published information or data regarding the timing and production of larvae and adults on each alternate host, mating behavior, origin of moth production (i.e., which alternate hosts) both locally and regionally, proximity of alternate host production to *Bt* cotton, survival and fecundity of each host, and fitness of adults coming off alternate hosts. Similarly, Dow AgroSciences should provide appropriate data regarding the effectiveness of supplemental insecticide treatment of *Bt* cotton fields to control putative resistant CBW. This research will improve the strength and reliability of an IRM plan to effectively reduce the likelihood that TBW, CBW, or PBW will become resistant to the Cry1Ac and Cry1F proteins.

Carbon isotope work by Gould et al. (2002) and Gore et al. (2003) indicates that a significant portion of the CBW population in *Bt* cotton areas arose from alternate hosts other than cotton. These findings support the importance of the non- *Bt* corn refuge in the Corn Belt. While alternate hosts should be considered when attempting to understand pest adaptation and resistance management, empirical evidence regarding their utilization and effective contribution to the production of SS moths to dilute resistance is not known. Dow AgroSciences makes certain assumptions regarding alternate hosts in its CBW modeling efforts. However, empirical data are needed to validate these assumptions. Further research is needed on the origin of the moths from

different alternate hosts throughout the growing season, mating dynamics, and fitness of the CBW moths emerging from different crops.

F. Public Interest Finding and Benefits Assessment

1. Public Interest Finding

To grant a conditional registration under Section 3(c)(7)(C) of FIFRA, EPA must determine that such conditional registration will, *inter alia*, be in the public interest. EPA determines whether conditional registration of a pesticide is in the public interest in accordance with the criteria set forth at 51 Fed. Reg. 7628 (*Conditional Registration of New Pesticides*, March 5, 1986). On the basis of analysis utilizing these criteria, EPA concludes that use of WideStrike®-protected cotton (EPA Reg. No. 68467-G) is in the public interest and supports the conditional registration of this pesticide under FIFRA section 3(c)(7)(C). EPA determines that use of WideStrike®-protected cotton will be in the public interest because it will result in a reduction of chemical insecticide use of higher risk insecticides (organophosphates, organochlorines, pyrethroids, and carbamates), and in direct and indirect human health and environmental benefits.

2. Benefits Assessment

a) Summary of Dow AgroSciences' Public Interest Document (Iamauti, 2003, MRID#458723-01)

Dow AgroSciences notes that cotton is the most important fiber crop in the U.S. and lepidopteran insects are the main insect problem for that crop. According to USDA/NASS data, 68% of all acres planted to cotton in the U.S. were treated with insecticides, accounting for over 21 million pounds of applied pesticide chemicals (NASS, 2002a). Insect control costs annually average $42 to over $100 per acre, or 13% to more than 31% of the total crop costs (approximately $320 per acre) (Global Insight, 2001).

Dow AgroSciences indicates that WideStrike® cotton offers the following benefits.

- In comparison to chemical insecticides, WideStrike® cotton offers growers in-plant control of insect damage that continues to be effective in inclement weather.
- WideStrike® cotton provides excellent control of TBW, CBW, and PBW at levels comparable or superior to current *Bt* cotton products on the market.
- WideStrike® cotton will bring additional control of other Lepidoptera (expanded target range including armyworms and loopers) to existing *Bt* cotton growers which will reduce insect control costs and simplify pest management in cotton.
- WideStrike® cotton has yields significantly greater than the yields of non-transgenic

varieties and will be achieved through lower costs of production.

- WideStrike® cotton will reduce insecticide use.
- WideStrike® cotton, with both the Cry1F and Cry1Ac proteins, will extend the durability of other *Bt* cotton products that express the Cry1Ac protein and will have greater durability than a single toxin product.
- Commercialization of WideStrike® cotton will increase the insect control options for cotton producers and expand the spectrum of insect control.
- WideStrike® cotton offers both human health and environmental benefits. Human health and environmental safety data for the Cry1F and Cry1Ac proteins indicates that risks to humans or non-targets posed by WideStrike® cotton expressing both the Cry1F and Cry1Ac proteins are minimal.

b) EPA Review

i) Economic Benefits

The yield performance information for WideStrike® cotton has been developed from 38 trials for efficacy, agronomic performance, and breeding traits used for line selection in 11 states over 2001 and 2002 (summarized in Appendix Table A.2 in Iamauti, 2003; MRID# 458723-01). Results from WideStrike® cotton yield trials indicate that (1) there is no direct deleterious effect of the genetic transformation on yield; (2) there is an indirect positive effect of the Cry1F/Cry1Ac ICP on yield through improved pest control; and (3) there may be potential for yield advantages to be combined with improved fiber quality to increase revenue from cotton, as a secondary benefit.

In addition to chemical insecticide use reduction of higher risk insecticides, the availability of WideStrike® cotton will expand the availability, variety, and durability of the available *Bt* PIP technologies, offer broad-spectrum lepidopteran protection for both primary and secondary, sporadic pests, increase cotton fiber quality because of the Acala background, and provide potential yield advantages for the grower. Growers will benefit from increased competition brought by the expanded number of *Bt* cotton variety choices when WideStrike® cotton is brought into the marketplace.

Results of trials where WideStrike® cotton was compared to non-*Bt* cotton demonstrate no negative impact on the yield of cotton in which Cry1F/Cry1Ac are expressed (MRID# 45808407). Trials were conducted in North Carolina, Louisiana, Mississippi, Alabama, Arizona, Arkansas, California, Georgia and Texas. Comparisons were made between WideStrike® and non-*Bt* cotton. Some trials included chemical insecticides. These trials measured the direct effect of the transgenes on yield. In these trials, the unsprayed WideStrike® cotton had yields that were statistically equal to or greater than the sprayed non-*Bt* control.

Agronomic characterization of cotton line MXB-13 established that fiber characteristics of MXB-13 are superior to those of the recurrent parent, PSC355. The MXB-13 (WideStrike®) line had a significantly greater fiber length, lower micronaire, and increased reflectance than the non-transgenic recurrent parent (PSC 355) . All three of these parameters contribute to a possibility for increased value of the fiber price that the grower would receive.

Dow AgroSciences' determined the gross economic value to the farmer from growing an WideStrike® cotton would be the combination of increased yield resulting from lepidopteran pest protection plus the possibility for increased value of the fiber quality. The gross yield value was calculated from the split-plot experiments with natural infestations and lint percent values from the agronomic trials to calculate lint yield per acre for each of the treatments. This was multiplied by the line price per pound as determined by the loan value (see Table 33 in Iamauti, 2003) to arrive at the gross crop value in dollars per acre **(see Table 5)**.

Table 5. Economic value of various line and treatment combinations (Reprinted from p. 63, Iamauti, 2003)

Line and Treatment	Lint Yield (lbs./acre)	Lint Value ($/lb)	Gross Crop Value ($/acre)
MXB-13/PSC355 Unsprayed	809	0.5504	445.27
Non-*Bt*/PSC355 Unsprayed	425	0.5130	218.03
Non-*Bt*/PSC355 Sprayed	760	0.5130	389.88

Dow AgroSciences' examined the potential economic benefits of WideStrike® cotton (with Cry1F and Cry1Ac expressed) across six market scenarios. The market scenarios are as follows: Southeast, Deep Mid-South, Northern Mid-South, California Acala, West Texas, Mid-South or SE, heavy pressure. The scenarios demonstrate the estimated losses due to lepidopteran pressure and are estimated on the basis of published data regarding sub-threshold losses and timing issues associated with spraying for insects. For each scenario comparisons are made for conventional cotton (non-Bt cotton receiving conventional chemical insecticides and managed for maximum yield), Bollgard cotton, and WideStrike® cotton. The treatment costs developed within each scenario include costs for chemicals, surfactant, application, and association activities such as scouting. The potential value attributable to fiber quality is not included in the values associated with each scenario described here.

Based on Dow AgroSciences' analysis, WideStrike®-protected cotton in comparison to conventionally managed non-*Bt* cotton lowered average costs for lepidopteran control from $3.06 per acre (West Texas, lowest pest pressure) to $73.20 per acre (Mid-South or Southeast under heavy Lepidoptera pressure). Average savings from the use of WideStrike®-protected cotton relative to Bollgard® cotton ranged from $3.83 per acre (West Texas) to $65.98 per acre

(California Acala). The Acala and Pima markets are unique new markets for *Bt* cotton products offered by WideStrike®-protected cotton.

EPA's analysis is based on the construction of a national demand curve based on estimates of the maximum benefit and maximum Bt related costs across all growers. The projected maximum benefits for WideStrike®-protected cotton is estimated to be $90 per acre. This is based on a maximum yield improvement of 12% and at $400 revenue per acre, a value of $48 per acre. Added to the benefits from yield improvement is the chemical cost savings. The maximum reduction of 4 to 5 treatments per year for bollworm/budworm and the additional secondary pests controlled by WideStrike®. At $8 to $10/ acre, the insecticide cost savings is in the $40 to $50 per acre range. This is based on a maximum yield improvement of 12%, and at $400 revenue per acre, a value of $48 per acre, and a maximum reduction of 5 treatments at $8.40 an acre which leads to a $42 per acre insecticide cost savings. Since the technology fee can be bundled into the cost of the regional seed variety, the registrant can vary the fee depending on the grower benefits. Depending to the extent to which price differentiation occurs, average net benefits per acre are likely to increase over that of Bollgard® cotton to between $4.49 (high differentiation) to $12.09 (low differentiation).

The simulated demand model is based on a Monte Carlo approach using a uniform distribution of benefits and *Bt*-related costs **(Table 6)**. The simulation model calculates adoption rate given a certain technology fee. *Bt*-related costs consist of refuge requirements and lack of marketability unique to genetically-modified organisms (GMO's), such as use in the organic cotton production. *Bt*-related costs are estimated to be $15/acre which is equivalent to a 91% adoption rate at a zero technology fee. The model would predict a technology fee in the $40 to $45 range based on revenue maximizing behaviors. This is a rough approximation to actual technology fees which are influenced by several other factors including competition with other pest control products for similar pest spectrum and efficacy to WideStrike® cotton. A factor working in the opposite direction is that seed varieties differ for each region. And because pest pressures and therefore benefits vary by region, the registrant can modify the fee accordingly. It is expected that areas with higher benefits would be charged a higher technology fee.

Table 6. Simulated Demand Curve for WideStrike®-Protected Cotton

Adoption Model Calc.	Tech Fee Schedule	Acres Adopted (millions)	Total Revenue (millions)	Marginal Revenue (millions)	Marginal Revenue per Acre	Marginal Adoption
0.085	75	1.06	79.69			1.06
0.19	65	2.38	154.38	74.69	56.90	1.31
0.31	55	3.88	213.13	58.75	39.17	1.50
0.42	45	5.25	236.25	23.13	16.82	1.38
0.47	40	5.88	235.00	(1.25)	(2.00)	0.63

Adoption Model Calc.	Tech Fee Schedule	Acres Adopted (millions)	Total Revenue (millions)	Marginal Revenue (millions)	Marginal Revenue per Acre	Marginal Adoption
0.53	35	6.63	231.88	(3.13)	(4.17)	0.75
0.59	30	7.38	221.25	(10.63)	(14.17)	0.75
0.64	25	8.00	200.00	(21.25)	(34.00)	0.63
0.76	15	9.50	142.50	(57.50)	(38.33)	1.50
0.91	0	11.38	0.00	(142.50)	(76.00)	1.88

Based on both EPA's and Dow's analyses, the average net economic benefits of WideStrike®-protected cotton increase over existing Bollgard® cotton.

ii) Efficacy and Comparative Product Performance

Dow AgroSciences conducted over 40 field trials in 2001 and 2002 to evaluate the efficacy of WideStrike® cotton (event MXB-13). These studies were performed in seven states and Puerto Rico under USDA APHIS notifications. Efficacy information was obtained on eight lepidopteran species including TBW, CBW, PBW, BAW, SAW, FAW, SL, and CL. These studies included artificial and natural infestations using split-plot design and randomized complete block designs as well as laboratory bioassays. Based on the efficacy data provided to EPA, WideStrike® cotton (MXB-13) provides excellent control of eight lepidopteran pests (TBW, CBW, PBW, FAW, BAW, SAW, SBL, and CL) common to commercial cotton crops in the U.S. A more detailed description of the methodology and full analysis of the WideStrike® (MXB-13) field efficacy studies is found in Pellow (2002, MRID# 458084-07). These studies were reviewed under separate cover by EPA and found to be "acceptable" (Matten, 2004a). Based on the efficacy studies and product comparisons summarized in Iamauti (2003), WideStrike® cotton provides excellent control of TBW, CBW, and PBW at levels comparable to, and in some cases, superior to, the *Bt* cotton products that are currently on the market. WideStrike® cotton offers the additional benefit of controlling occasional pests of cotton, including armyworms and loopers, which also contribute to reduced yield in cotton. WideStrike® cotton performed as well or significantly better under heavy natural infestations without the use of chemical sprays.

iii) Human Health and Environmental Benefits

WideStrike® cotton adoption has direct benefits to human health and environmental safety. WideStrike® cotton has better human health and environmental safety profiles than any competitive chemical insecticide and is comparable to that of other *Bt* cottons. WideStrike®

cotton poses negligible concerns to workers, by-standers, and non-target organisms.

iv) Insecticide Use Reduction

Adoption of WideStrike® cotton is expected to provide substantial direct human health and environmental benefits by reducing applications of chemical insecticides to control pests in field cotton. Dow estimates that for each percent of planted cotton acres where WideStrike® cotton is adopted (for acres not previously planted to Bollgard® cotton), there will be an annual reduction in approximately 32,000-pounds (16 tons) active ingredient of the competitive pesticides. In those cases where WideStrike® replaces Bollgard® cotton, the insecticide use reduction will be closer to the 15,880 lbs/yr, reflecting reduction in spray frequency due to a broader pest control spectrum. Doane market research data through 2003 support the continued insecticide use reduction of Bollgard® cotton. Further reductions of insecticide use through the additional pests controlled are expected, but they will be substantially less than those just for the budworm/budworm complex because these pests do not receive as much chemical treatment as the bollworm/budworm complex. Additional indirect economic and environmental benefits are expected to be a consequence of reduced chemical insecticide use with adoption of WideStrike® cotton. These benefits range from reduced energy consumption for manufacture, transport, and application of chemical insecticides to reduced waste streams arising from pesticide manufacture and the disposal of waste products such as containers and residuals from application. Energy savings per each one percent of acres planted to WideStrike® cotton (annually) is estimated to be $3.25 \text{ X } 10^{10}$ BTU or about 5,610 barrels of crude oil.

With use of WideStrike® cotton, chemical insecticide treatments are expected to be reduced in new cotton areas which adopt Bt crop technology for the first time. In addition , for areas already using Bt crop technology but also suffering loses from armyworms, loopers, cutworms, and other secondary pests controlled by Cry1Ac and Cry1F combination, further reduction in chemical insecticidal use is expected. These reductions in pesticide use will not be to the extent of the reductions brought on by reducing chemical controls to the budworm/bollworm complex. Current chemical treatments directed toward armyworm, loopers and cutworms are small in comparison to the budworm/bollworm complex, but still additional chemical treatment reductions (approximately 17%) are expected with the introduction of WideStrike® cotton. The efficacy of WideStrike®-protected cotton against CBW will also result in additional insecticide use reductions.

With WideStrike® protected cotton, a maximum reduction of 5 insecticide treatments per year at $8.40 an acre is expected in the Deep South, where there is maximum pest pressure due to TBW and CBW.

v) Insect Resistance Management Benefits

Dow AgroSciences' proposed product IRM plan (Storer, 2002; MRID# 45808415) provides a detailed analysis of the durability of WideStrike® cotton. EPA has reviewed Dow's proposed product durability/insect resistance management (IRM) plan for WideStrike® (Storer, 2002;

MRID# 45808415) and found it "acceptable" (Matten, 2004). EPA agrees with the Dow AgroSciences' conclusion that WideStrike® cotton, with Cry1F and Cry1Ac, will extend the durability of other *Bt* cotton products and will have greater durability than a single toxin product that targets TBW and CBW. Resistance has been observed to pyrethroids in TBW, and to a small extent, there have been changes in susceptibility to pyrethroids in CBW. WideStrike® cotton, with both the Cry1F and Cry1Ac ICPs, will extend the durability of other *Bt* cotton products that express the Cry1Ac ICP and will have greater durability than a single toxin product. Deployment of two ICPs means that TBW and CBW will have to be resistant to both the Cry1Ac and Cry1F proteins in order to survive on WideStrike® cotton. Laboratory- and field-selected resistance to *Bt* toxins has not been correlated with resistance to other insecticides used in cotton, including pyrethroids, organophosphates, carbamates, and spinosad. The addition of WideStrike®-protected cotton will provide the grower with additional control options that will likely reduce the selection pressure resistance to the other technologies (chemical insecticides and *Bt* cottons), and thus contribute to the sustainability of all of these technologies.

G. Response to June 8-10, 2004 Scientific Advisory Panel Meeting Report

The Agency asked a subpanel of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), Scientific Advisory Panel (SAP) several questions on scientific issues pertaining to its review of product characterization, human health risk, ecological risk, and insecticide resistance management of *Bt* cotton products including WideStrike® (Cry1Ac/Cry1F) cotton. The review was conducted in an open Panel meeting held in Arlington, Virginia, from June 8-10, 2004. The SAP meeting response was finalized August 19, 2004 and is available at http://www.epa.gov/pesticides/scipoly/June. The Agency's response to the SAP report is provided below.

Human Health Risk and Product Characterization

The Agency asked the Panel to comment on the safety of the introduced Cry1Ac and Cry1F protein insecticidal toxins, and asked the panel to identify instances where it would be justified to require the toxicity testing of two proteins in combination.

The Panel agreed with the Agency that testing for interaction between the proteins is not justified unless specific evidence points to the contrary. The Panel is not aware of any instances where a "new" toxin has been created by unexpected interaction between two known proteins. Although there are many examples of binary (or multimeric) protein toxins, the interactions between the proteins involved are specific among members of a toxin family, and it appears unlikely that unexpected interactions will occur between two unrelated proteins. The only examples would be if physical interactions between two unstable proteins inhibited gastric digestion or increased stability during processing. But these situations are not present in the case of WideStrike® cotton.

Ecological Risk

The Panel was asked to comment on the need for non-target hazard data development on the combinations of Cry proteins being considered for registration when data on the effects of the individual Cry proteins are readily available and show no adverse effects.

According to the Panel, direct hazards to vertebrates due to exposure to the Cry1F and Cry1Ac proteins should be minimal or non existent, making consideration of synergy in vertebrates unwarranted.

Insect Resistance Management

The Agency asked the SAP six questions regarding its scientific analysis of the insect resistance management plan for Dow AgroSciences' WideStrike® cotton. The Agency's response to the SAP report on these issues is provided below.

1. Dose. The Agency asked the SAP to comment on the Agency's analysis of dose for TBW, CBW, and PBW, the likelihood that resistance will be inherited as a recessive trait, and its impact on insect resistance management for WideStrike® cotton. The SAP's response:

> " The combined expression of *Bt* proteins in WideStrike® cotton meets the Agency's definitions of high dose for pink bollworm (PBW) and tobacco budworm (TBW). In addition, reasonable doses of the combined protein were evident for control of cotton bollworm (CBW). Based on the high dose evidence, the Panel concluded that it is valid to assume that resistance occurring in PBW or TBW will likely be inherited as a recessive trait. However, CBW is more tolerant of both proteins and it seems possible that resistance will be less recessive. WideStrike® cotton does appear to offer a high dose for TBW, a high dose of Cry1Ac for PBW, and reasonable doses of Cry1F and Cry1Ac for CBW. The same high dose/refuge strategy practiced thus far as a resistance management approach for Bollgard® cotton should be applied for WideStrike®."

The SAP agreed with the Agency's analysis of dose for TBW, CBW, and PBW, the likelihood that resistance will be inherited as a recessive trait, and its impact on insect resistance management for Dow AgroSciences, WideStrike® cotton. No additional data on dose are necessary.

2. Cross-resistance. The Agency asked the SAP to comment on EPA's conclusion that incomplete shared binding of Cry1Ac and Cry1F receptors, in TBW and CBW, is expected to lead to incomplete cross-resistance and thus the likelihood of enhanced survival on WideStrike® cotton is expected to be small. Also, the Panel was requested to comment on EPA's conclusion that resistance is more likely to be associated with receptor binding modifications rather than other mechanisms of resistance such as detoxification in the midgut lumen by proteases that cleave the insecticidal control protein(s), metabolic adaptations, protease inhibition, gut recovery, and behavioral adaptations. The SAP's response:

> "While the Panel supported the Agency's conclusion that incomplete shared binding of Cry1Ac and Cry1F receptors in TBW and CBW is expected to lead to incomplete cross-

resistance, differences were expressed on the molecular mechanism involved in the process. In addition, the Panel raised the issue that another as yet unidentified major resistance mechanism may occur. The Panel agreed with the Agency that there is no basis to believe that the occurrence of resistance in the field will be due to a mechanism other than binding site modification."

The SAP agreed with the Agency's analysis that there will be incomplete cross-resistance. The Agency recognizes the SAP's concern that there might be yet another unidentified major resistance mechanism, but it still maintains, as does the SAP, that the likely mechanism of resistance in the field will be binding site modification. EPA appreciates the SAP's analysis of the uncertainties associated with receptor binding studies and will be cognizant of these uncertainties as it reviews other receptor binding studies used to describe or predict the likelihood of cross-resistance.

3. CBW modeling. The Agency asked the SAP to comment on the predictions made by the Dow AgroSciences' CBW model, i.e., the likelihood that the population fitness of CBW on WideStrike® cotton in a 15-year time horizon will remain unchanged, even without a high dose for either Cry1Ac or Cry1F and incomplete cross-resistance (60% of Cry1Ac binds to the Cry1F receptor). The SAP's response:

"The Panel identified several areas of concern with the Dow Agrosciences CBW model that make its use problematic. These problems must be addressed if this model is to be used to assess the durability of WideStrike® cotton. The Panel believed that use of the current model, once corrected of the identified errors, would be an appropriate vehicle to explore the parameter space with the goal of finding areas where resistance does occur in the 15 year time horizon and assessing whether it occurs within biologically plausible initial conditions and parameter values."

The SAP identified the following areas of concern: interpretation of factor Z, calculations of expected genotype mortalities, choice of population fitness rather than allele frequency as output, appearance of low selection pressure making the Dow AgroSciences' model overly optimistic, and immigration of moths to the Delta region. The Agency does not fully agree with the Panel's conclusion that there are several areas of concern or errors in Dow's model, but does agree with the Panel's overall conclusion that the durability of WideStrike® cotton is at least 15 years. A response by Dr. Nicholas Storer, Dow Agrosciences, e-mail dated June 14, 2004 which was given to the SAP, provides a counter explanation to address each of the SAP's concerns which seem as valid as the explanation provided by the SAP. Further discussion of these issues is recommended. The Agency believes that Dow AgroSciences' spatially-explicit model offers a simplified version of reality that provides insight into the factors involved in CBW resistance evolution and how they interact. While there may be differences in what assumptions should be made in the model, the model's input parameters, and how the output should be expressed that may require further discussion, the overall conclusions of this model still hold true. That is, the two *Bt* proteins expressed in WideStrike® cotton, Cry1F and Cry1Ac, have multiple binding-sites in which a limited few are shared, but most are unique; cross-resistance will be incomplete and thus resistance to either or both toxins will be slowed; and natural and structured refugia exist for CBW across its host range (although further empirical data or studies from the published literature are recommended to be provided to the Agency. This combination leads to an

expectation of slow resistance development compared with a single binding site and only a single *Bt* protein. As the Agency has written in its analysis provided to the SAP, earlier modeling efforts have predicted that the durability of a two-gene pyramid will always be greater than a single-gene product. Dow AgroSciences' model supports the same conclusions as derived from the previous cited modeling efforts. Dow AgroSciences' efforts indicate that WideStrike® (a pyramid for TBW and CBW) will have predicted advantages over a single protein product even when there is some cross-resistance and when there is somewhat less than a high dose for either protein. Again, the SAP agrees with this conclusion. While the Agency does not fully agree with the Panel's analysis regarding the Dow AgroSciences model itself, it believes that the Panel's advice is valuable and insightful. Therefore, because there is doubt as to the acceptability of the Dow AgroSciences model in its current form, it is recommended that if Dow AgroSciences wishes to use this model in the future to support CBW resistance management options then it should consider the recommendations of the SAP and revise this model. In spite of the shortcomings of the CBW model cited by the Panel, the Panel still agreed with the Agency's analysis that the durability of WideStrike® cotton is longer than 15 years. It is this conclusion that is the most important regardless of the assumptions and interpretation of the Dow AgroSciences' CBW model.

4. TBW modeling. The Agency asked the SAP to comment on the relative WideStrike® cotton durability against TBW using the Peck et al. (1999) model. The SAP's response:

> "Since the dose of the Cry1Ac and Cry1F in WideStrike® Cotton was demonstrated to be high against populations of TBW, the Panel believed that WideStrike® will be more durable than that predicted by Peck (1999) for single Cry1Ac cotton."

The SAP agreed with the Agency's analysis of TBW durability. That is, WideStrike® cotton durability against TBW will be greater for this two toxin, high dose product than predicted by Peck et al. (1999) for a *Bt* cotton expressing a single toxin, Cry1Ac. No additional TBW modeling is necessary.

5. Alternate hosts. The Agency asked the SAP to comment on the following:
a) the sufficiency of the WideStrike® cotton database to address the issue of CBW alternate hosts as natural refugia, and, b) whether additional data are needed on the larval and adult production of CBW on each alternate host for each generation relative to cotton and WideStrike® cotton and the spatial scale and source of moth production to confirm the effectiveness of CBW alternate hosts as natural refugia. The SAP's response:

> "The Panel agreed that the HOSTS database is insufficient to address the issue of CBW alternate hosts as natural refugia. The Panel agreed that there are insufficient empirical data in the registrant report to demonstrate that alternative hosts are producing susceptible, fit individuals in sufficient quantity, at the correct time and proximity to maximize the probability of matings between homozygous-susceptible individuals and individuals heterozygous for resistant traits."

The SAP agreed with the Agency's analysis that the database provided by Dow AgroSciencesis insufficient to support the use of CBW alternate hosts as natural refugia. Based on the recommendation of the SAP, it is recommended that Dow AgroSciences provide the Agency with additional empirical data or published literature (other than Gould et al., 2002) that support the

use of CBW alternate hosts as natural refugia. Data would include larval and adult production of CBW on each alternate host for each generation relative to cotton and WideStrike® cotton and the spatial scale and source of moth production.

6. IRM Plan. The Agency asked the SAP to comment on the scientific data available to support the proposed IRM plan and whether that data support a delay in resistance of TBW, CBW, and PBW to the Cry1F and Cry1Ac proteins expressed in WideStrike® cotton for at least 15 years. The SAP's response:

> "Even though the Panel raised limitations with the model (note: CBW), the Panel was in strong agreement that the proposed IRM plan by the registrant is sufficient for WideStrike® cotton and supported the prediction of a delay in resistance of TBW, CBW and PBW to WideStrike® cotton for 15 years. The overall consensus was that the existing IRM options that have been applied to the single-toxin Bollgard® cotton will be equally or even more effective in protecting against resistance in the double-toxin WideStrike® cotton."

The Panel agreed with the Agency's analysis that the proposed IRM is acceptable and will likely delay TBW, CBW, and PBW to the toxins expressed in WideStrike® cotton for at least 15 years.

III. Terms and Conditions of Registration

Dow AgroSciences is required to do the following as terms and conditions of the registration.

1) This FIFRA Section 3(c)7(C) conditional registration will automatically expire on September 30, 2009.

2) The subject registration will be limited to *Bacillus thuringiensis* Cry1Ac (synpro) and Cry1F (synpro) proteins and the genetic material necessary for their production in or on cotton.

3) Submit/cite all required data required for registration of your product under FIFRA Section 3(c)5 when the Agency requires registrants of similar products to submit such data.

4) Submit production information for this product to Mr. Owen Beeder of the Office of Pesticide Programs, Registration Division (mail code 7505C) for the fiscal year in which this product is conditionally registered., in accordance with FIRA Section 29. The fiscal year begins October 1 and ends September 30. Production information will be submitted to the Agency no later than December 15, following the end of the preceding fiscal year.

5) By July 31, 2005, Dow AgroSciences must provide the Agency with additional empirical data or published literature (other than Gould et al., 2002) that supports the use of CBW alternate hosts as natural refugia. Data would include larval and adult production of CBW on each alternate host for each generation relative to cotton and WideStrike® cotton and the spatial scale and source of moth production.

6) Should Dow AgroSciences wish to use their CBW resistance management model to support changes to the insect resistance management strategy, then this model must be revised per the recommendations of the June 8-10, 2004 SAP report dated August 19, 2004 and submitted to the

Agency for review.

7) Dow AgroSciences must submit an avian chronic exposure study by September 30, 2008.

8) Dow AgroSciences must submit a non-target insect more appropriate for cotton fields, i.e., a maximum hazard dose laboratory toxicity study using the organism, Orius insidiosus (minute pirate bug) by September 30, 2008.

9) Dow AgroSciences must submit soil fate/terrestrial expression studies for long range soil persistence by September 30, 2008.

10) Gene Flow: The following information regarding commercial production must be included in the grower guide for WideStrike® Cotton and is a term of this amendment:

a) No planting of WideStrike® cotton is permitted south of Route 60 (near Tampa) in Florida.
b) Commercial culture of WideStrike® cotton is prohibited in Hawaii, Puerto Rico, and the US Virgin Islands.

The following information regarding test plots and seed production must occur on bags of WideStrike® cotton intended for these purposes and is a term of this amendment.

a) Test plots or breeding nurseries, regardless of the plot size, established in Hawaii must not be planted within 3 miles of *Gossypium tomentosum* and must be surrounded by 24 border rows of a suitable pollinator trap crop.

b) Experimental plots and breeding nurseries of Bt.-cotton are prohibited on the U.S. Virgin Islands, and

c) Test plots or breeding nurseries, regardless of the plot size, established on the island of Puerto Rico must not be planted within 3 miles of feral cotton plants and must be surrounded by 24 border rows of a suitable pollinator trap crop.

Upon approval by EPA, test plots and/or breeding nurseries in Hawaii, the U.S. Virgin Islands, and Puerto Rico may be established without restrictions if alternative measures, such as insecticide applications, are shown to effectively mitigate gene flow.

11) Insect Resistance Management

Dow AgroSciences must implement the following IRM program:

- Requirements relating to creation of a non-*Bt* cotton refuge in conjunction with the planting of any acreage of *Bt* cotton;

- Requirements for Dow AgroSciences to prepare and require *B*t cotton users to sign

"grower agreements" which impose binding contractual obligations on the grower to comply with the refuge requirements;

- Requirements for Dow AgroSciences to develop, implement, and report to EPA on programs to educate growers about IRM requirements;

- Requirements for Dow AgroSciences to develop, implement, and report to EPA on programs to evaluate and promote growers' compliance with IRM requirements;

- Requirements for Dow AgroSciences to develop, implement, and report to EPA on programs to evaluate whether there are statistically significant and biologically relevant changes in susceptibility to the Cry1Ac and Cry1F proteins in the target insects;

- Requirements for Dow AgroSciences to develop, and if triggered, to implement a "remedial action plan" which would contain measures Dow AgroSciences would take in the event that any insect resistance was detected as well as to report on activity under the plan to EPA;

- Annual reports on or before January 31st each year.

a. Refuge Requirements

All growers of WideStrike® cotton must employ one of the following structured refuge options:

1) External, Unsprayed Refuge

Ensure that at least 5 acres of non-*Bt* cotton (refuge cotton) is planted for every 95 acres of WideStrike® cotton. The size of the refuge must be at least 150 feet wide, but preferably 300 feet wide. This refuge may not be treated with sterile insects, pheromone, or any insecticide (except listed below) labeled for the control of tobacco budworm, cotton bollworm, or pink bollworm. At the pre-squaring cotton stage only, the refuge may be treated with any lepidopteran insecticide to control foliage feeding caterpillars. The refuge may be treated with acephate or methyl parathion at rates which will not control tobacco budworm or the cotton bollworm (equal to or less than 0.5 lbs active ingredient per acre). The variety of cotton planted in the refuge must be comparable to WideStrike® cotton, especially in the maturity date, and the refuge must be managed (e.g., planting time, use of fertilizer, weed control, irrigation, termination, and management of other pests) similarly to WideStrike® cotton. Ensure that a non-*Bt* cotton refuge is maintained within at least ½ linear mile (preferably adjacent to or within 1/4 mile or closer) from the *Bt* cotton fields.

2) External Sprayed Refuge

Ensure that at least 20 acres of non-*Bt* cotton are planted as a refuge for every 80 acres of WideStrike® cotton (total of 100A). The variety of cotton planted in the refuge must be comparable to *Bt* cotton, especially in the maturity date, and the refuge must be managed (e.g., planting time, use of fertilizer, weed control, irrigation, termination, and management of other

pests) similarly to WideStrike® cotton. The non-*Bt* cotton may be treated with sterile insects, insecticides (excluding foliar *Btk* products), or pheromones labeled for control of the tobacco budworm, cotton bollworm, or pink bollworm. Ensure that a non-*Bt* refuge is maintained within at least 1 linear mile (preferably within ½ mile or closer) from the *Bt* cotton fields.

3) Embedded Refuge

Plant at least 5 acres of non-*Bt* cotton (refuge cotton) for every 95 acres of WideStrike® cotton. The refuge cotton must be embedded as a contiguous block within the Bt cotton field, but not at one edge of the field (i.e., refuge block(s) surrounded by WideStrike® cotton). For very large fields, multiple blocks across the field may be used. For small or irregularly shaped fields, neighboring fields farmed by the same grower can be grouped into blocks to represent a larger field unit, provided the block exists within one mile squared of the WideStrike® cotton and the block is at least 150 feet wide, but preferably 300 feet wide. Within the larger field unit, one of the smaller fields planted to non-*Bt* cotton may be utilized as the embedded refuge. The variety of cotton planted in the refuge must be comparable to WideStrike® cotton, especially in the maturity date, and the refuge must be managed (e.g., planting time, use of fertilizer, weed control, irrigation, and management of other pests) similarly to WideStrike® cotton. This refuge may be treated with sterile insects, any insecticide (excluding foliar *Btk* products), or pheromone labeled for the control of tobacco budworm, cotton bollworm, or pink bollworm whenever the entire field is treated. The refuge may not be treated independently of the surrounding WideStrike® cotton field in which it is embedded (or fields within a field unit) except only at the pre-squaring cotton stage when the refuge may be treated with any lepidopteran insecticide to control foliage feeding caterpillars..

4) Embedded Refuge for Pink Bollworm Only

Plant the refuge cotton as at least one single non-*Bt* cotton row for every six to ten rows of WideStrike® cotton. The refuge may be treated with sterile insects, any insecticide (excluding foliar *Btk* products), or pheromone labeled for the control of pink bollworm whenever the entire field is treated. The in-field refuge rows may not be treated independently of the surrounding *Bt* cotton field in which it is embedded. The refuge must be managed (fertilizer, weed control, etc.) identically to the WideStrike® cotton. There is no field unit option.

5) Community Refuge

This option allows multiple growers to manage refuge for external, unsprayed and external, sprayed refuge options or both. This option is not allowed for the embedded/in-field options. The community refuge for insect resistance management must meet the requirements of either the 5% external unsprayed refuge and/or the 20% sprayed option, or an appropriate combination of the two options. The community refuge pilot must consist of the following:

> There will be a community refuge coordinator for each pilot site. Each community refuge coordinator must submit a signed community refuge form listing all of the participants at the pilot site to Dow AgroSciences by July 1st annually. Dow AgroSciences must provide EPA, if requested, with a copy of the signed community refuge form. The community

refuge coordinator will maintain a copy of the field map (to scale) or suitable scalar representation of the community refuge for review by Dow AgroSciences or EPA as part of the compliance program.

On an annual basis, Dow AgroSciences must conduct at least one telephone audit of a statistically representative sample of community refuge coordinators from communities in all states participating in the community refuge. EPA shall review the questions annually prior to the start of the growing season.

The community refuge program users must be included in the telephone compliance survey and the on-farm visits to be conducted by Dow AgroSciences under section 3.c. below.

Beginning January 31, 2006 and annually each January 31st, Dow AgroSciences must provide a written report to EPA annually on community refuge use and compliance. The community refuge report may be combined in a single report with other compliance activities.

On an annual basis, Dow AgroSciences must conduct a review of the community refuge program and submit that review to the Agency as to any proposed changes by January 31st. An appropriate amendment for any proposed changes must be submitted to the Agency.

b. Grower Agreements

While Dow AgroSciences will have flexibility to design its program to fit its own business practices, the registration is specifically conditioned on meeting the following requirements.

1) Persons purchasing the *Bt* cotton product must sign a grower agreement. The term "grower agreement" refers to any grower purchase contract, license agreement, or similar legal document.

2) The grower agreement and/or specific stewardship documents referenced in the grower agreement must clearly set forth the terms of the current IRM program. By signing the grower agreement, a grower must be contractually bound to comply with the requirements of the IRM program.

3) Dow AgroSciences must establish by the 2005 growing season, a system which is reasonably likely to assure that persons purchasing the *Bt* cotton product will affirm annually that they are contractually bound to comply with the requirements of the IRM program. The proposed system will be submitted to EPA on or before December 1, 2004.

4) Dow AgroSciences must submit a copy of its grower agreement to EPA by December 1, 2004. If Dow AgroSciences wishes to change any part of the grower agreement that would affect either the content of the IRM program or the legal enforceability of the provisions of the agreement relating to the IRM program, thirty days prior to implementing a proposed change, Dow

AgroSciences must submit to EPA the text of such changes to ensure the agreement is consistent with the terms and conditions of this amendment.

5) Dow AgroSciences must establish a system which is reasonably likely to assure that persons purchasing the *Bt* cotton sign grower agreement(s), and must provide by December 1, 2004 a written description of that system.

6) Dow AgroSciences shall maintain records of all *Bt* cotton grower agreements for a period of three years from December 31 of the year in which the agreement was signed.

7) Beginning on January 31, 2006 and annually thereafter, Dow AgroSciences shall provide EPA with a report on the number of units of the *Bt* cotton seed shipped and not returned and the number of such units that were sold to persons who have signed grower agreements. The report shall cover the time frame of the twelve-month period covering the prior October through September. Note: the first report shall contain the specified information for the time frame starting with the date of registration and ending September 30, 2005.

8) Dow AgroSciences must allow a review of the grower agreements and grower agreement records by EPA or by a State pesticide regulatory agency if the State agency can demonstrate that the names, personal information, and grower license number will be kept as confidential business information.

c. IRM Education and IRM Compliance Monitoring Programs

Dow AgroSciences must implement the following IRM education and compliance monitoring programs:

1) Dow AgroSciences must design and implement a comprehensive, ongoing IRM education program designed to convey to *Bt* cotton users the importance of complying with the IRM program. The program shall include information encouraging *Bt* cotton users to pursue optional elements of the IRM program relating to refuge configuration and proximity to *Bt* cotton fields. The education program shall involve the use of multiple media, e.g. face-to-face meetings, mailing written materials, and electronic communications such as by internet or television commercials. Copies of the materials, including the Grower Guide or other technical bulletins, must be submitted to EPA for their records. The program shall involve at least one written communication annually to each WideStrike® cotton grower separate from the grower agreement. Dow AgroSciences shall coordinate its education program with educational efforts of other organizations, such as the National Cotton Council and state extension programs.

2) Annually, Dow AgroSciences shall revise, and expand as necessary, its education program to take into account the information collected through the compliance survey required under paragraph 6 and from other sources. The changes shall address aspects of grower compliance that are not sufficiently high.

3) Beginning January 31, 2006 and annually thereafter, Dow AgroSciences shall provide a report to EPA summarizing the activities it carried out under its education program for the prior year and

its plans for its education program during the current year.

4) Dow AgroSciences shall design and implement an ongoing IRM compliance assurance program designed to evaluate the extent to which growers are complying with the IRM program and that takes such actions as are reasonably needed to assure that growers who have not complied with the program either do so in the future or lose their access to the *Bt* cotton product. Dow AgroSciences shall prepare and submit by January 31, 2005 a written description of its compliance assurance program. Other required features of the program are described in paragraphs 5 - 12 below.

5) Dow AgroSciences shall establish and publicize a "phased compliance approach," i.e., a guidance document that indicates how Dow AgroSciences will address instances of non-compliance with the terms of the IRM program and general criteria for choosing among options for responding to any non-compliant growers. The options shall include withdrawal of the right to purchase WideStrike® cotton for an individual grower or for all growers in a specific region. An individual grower found to be significantly out of compliance two years in a row would be denied sales of the product the next year.

6) The IRM compliance assurance program shall include an annual survey of a statistically representative sample of WideStrike® cotton growers conducted by an independent third party. The survey shall measure the degree of compliance with the IRM program by growers in different regions of the country and consider the potential impact of non-response. Dow AgroSciences shall provide a written summary of the results of the prior year's survey to EPA by January 31st of each year. Dow AgroSciences shall confer with EPA on the design and content of the survey prior to its implementation for the 2005 growing season and annually, thereafter.

7) Annually, Dow AgroSciences shall revise, and expand as necessary, its compliance assurance program to take into account the information collected through the compliance survey required under paragraph 6] and from other sources. The changes shall address aspects of grower compliance that are not sufficiently high. Dow AgroSciences will confer with the Agency prior to adopting any changes.

8) Dow AgroSciences shall train its representatives who make on-farm visits with WideStrike® cotton growers to perform assessments of compliance with IRM requirements. In the event that any of these visits results in the identification of a grower who is not in compliance with the IRM program, Dow AgroSciences shall take appropriate action, consistent with its "phased compliance approach," to promote compliance.

9) Dow AgroSciences shall carry out a program for investigating "tips and complaints" that an individual grower or growers is/are not in compliance with the IRM program. Whenever an investigation results in the identification of a grower who is not in compliance with the IRM program, Dow AgroSciences shall take appropriate action, consistent with its "phased compliance approach."

10) If a grower, who purchases WideStrike® cotton for planting, was specifically identified as not being in compliance during the previous year, Dow AgroSciences shall visit the grower and

evaluate whether that the grower is in compliance with the IRM program for the current year.

11) Beginning January 31, 2006 and annually thereafter, Dow AgroSciences shall provide a report to EPA summarizing the activities it carried out under its compliance assurance program for the prior year and its plans for its compliance assurance program during the current year. Included in that report will be the percent of growers using each refuge option (or combination of options) by region, the approximate number or percent of growers visited on farm by Dow AgroSciences and the results of these visits the number of tips investigated, the percent of growers not in compliance with each refuge option (both size and distance), and the follow-up actions taken.

12) Dow AgroSciences must allow a review of the compliance records by EPA or by a State pesticide regulatory agency if the State agency can demonstrate that the names, personal information, and grower license number of the growers will be kept as confidential business information.

d. Insect Resistance Monitoring.

The registration of Cry1Ac and Cry 1F PIPs expressed in cotton is conditioned on Dow AgroSciences carrying out appropriate programs to detect the emergence of insect resistance as early as possible. Resistance monitoring programs include: surveying insects for potential resistance and collection of information from growers about events that may indicate resistance. Dow AgroSciences should coordinate its monitoring efforts for WideStrike® with the current resistance monitoring programs for other *Bt* ICPs. The Agency is imposing the following conditions:

1) Dow AgroSciences will develop and ensure the implementation of a plan for resistance monitoring for *Heliothis virescens* (tobacco budworm) and *Helicoverpa zea* (cotton bollworm). The plan shall include provision for conducting annual studies to evaluate any potential change in susceptibility of tobacco budworm and cotton bollworm population to Cry1Ac and Cry1F proteins. Sites must be focused in areas with high risk of resistance (e.g. where adoption is at least 75% of the cotton planted in that county or parish) while overall being distributed throughout the areas where tobacco budworm and cotton bollworm are important pests. The sampling program should be segregated into different sampling regions rather than sampling within each state in which these insects are economic pests At least 20 specific collection sites will be established in time for the 2005 growing season. Discriminating doses for each toxin must be developed for tobacco budworm and cotton bollworm. Dow AgroSciences must provide EPA with the baseline susceptibility data for the Cry1F and Cry1Ac proteins and establish diagnostic/discriminating dose concentrations for both the Cry1F and Cry1Ac proteins by September 1, 2005.

2) Dow AgroSciences will develop and ensure the implementation of a plan for resistance monitoring for *Pectinophora gossypiella* (pink bollworm). The plan shall include provision for conducting annual studies to evaluate any potential change in susceptibility of pink bollworm population to Cry1Ac and Cry 1F proteins. Collection sites must be focused in areas of high adoption, with the goal of including all states where pink bollworm is an economic pest. Dow

AgroSciences must provide EPA with the baseline susceptibility data for the Cry1Ac protein and establish a diagnostic/discriminating dose concentration for both the Cry1Ac proteins by September 1, 2005.

3) Dow AgroSciences shall provide a detailed description to EPA of its resistance monitoring plan by January 31, 2005. The description shall include: sampling (number of locations and samples per locations), sampling methodology, bioassay methodology, standardization procedures, detection technique and sensitivity, and the statistical analysis of the probability of detecting resistance.

4) Dow AgroSciences must also follow up on grower, extension specialist or consultant reports of less than expected results or control failures (such as increases in damaged squares or bolls) for the target lepidopteran pests (*Heliothis virescens* (TBW) and *Helicoverpa zea* (CBW), *Pectinophora gossypiella* (PBW)) as well as for cabbage looper, soybean looper, , fall armyworm, southern armyworm, and beet armyworm. Dow AgroSciences will instruct its customers (growers and seed distributors) to contact them (e.g., via a toll-free customer service number) if incidents of unexpected levels of tobacco budworm, cotton bollworm, or pink bollworm damage occur. Dow AgroSciences will investigate all damage reports. See Remedial Action Plans section below.

5) A report on results of resistance monitoring and investigations of damage reports must be submitted to the Agency annually by September 1st each year for the duration of the conditional registration.

e. Remedial Action Plans

Specific remedial action plans are required for WideStrike® cotton for the purpose of containing resistance and perhaps eliminating resistance if it develops. One remedial action plan is for the area where pink bollworm is the predominate pest and the other is for the area where tobacco budworm and cotton bollworm are the predominate pests.

1) Remedial Action Plan for Pink Bollworm

If resistance involves the pink bollworm (*Pectinophora gossypiella*), Dow AgroSciences must implement the Arizona *Bt* Cotton Working Group's Remedial Action Plan. Dow AgroSciences must obtain approval from EPA before modifying the Arizona *Bt* Cotton Working Group's Remedial Action Strategy. The Arizona *Bt* Cotton Working Group's Remedial Action Plan can be found in Appendix 1.

2) Remedial Action Plan for Tobacco Budworm and Cotton Bollworm

Based upon the Arizona model, a Remedial Action Plan for cotton bollworm and tobacco budworm must be developed and implemented by Dow AgroSciences if suspected or confirmed resistance is found. Dow AgroSciences must submit a remedial action plan (or plans) for tobacco budworm and cotton bollworm to the Agency by January 31, 2006 for approval prior to its implementation. An Interim Remedial Action Plan for Cotton Bollworm and Tobacco Budworm

is contained in Appendix 2. Dow AgroSciences must obtain approval from EPA before modifying the Remedial Action Plan for Cotton Bollworm and Tobacco Budworm.

Annual Reports

Dow AgroSciences will provide an annual report to EPA on its Cry1Ac and Cry1F PIPs expressed in cotton on or before January 31st each year. This report must include, but is not limited to, annual sales (both units sold and estimated acres planted) by county and by state (sales data must be summed individually for each state), research status for any outstanding data requirements as covered in 3 above, grower education completed last year and planned for the following year with any changes highlighted, the description of grower agreements in place, grower compliance with IRM requirements including compliance with the community refuge option, and insect resistance monitoring results.

12) If these conditions are not complied with, the registration will be subject to cancellation in accordance with FIFRA sec. 6(e). Your release for shipment of the product constitutes acceptance of these conditions.

IV. Regulatory Position

Pursuant to FIFRA section 3(c)(7)(C), EPA may conditionally register a new pesticide active ingredient for a period of time reasonably sufficient for the generation and submission of required data that are lacking because insufficient time has elapsed since the imposition of the data requirement for those data to be developed. EPA may grant such conditional registration only if EPA determines that (1) the use of the pesticide product during the period of the conditional registration will not cause any unreasonable adverse effect on the environment, and (2) the registration and use of the pesticide during the conditional registration is in the public interest. EPA determines that all of these criteria have been fulfilled.

The first criterion under FIFRA section 3(c)(7)(C) mentioned above has been met since insufficient time has elapsed since the imposition of the data requirements for:

1) A six week broiler dietary study to assess hazard to wild and domesticated fowl from chronic exposure to Cry1F/Cry1Ac protein

2) A laboratory toxicity test with *Orius insidiosus* (insidious flower bug).

3) Field degradation studies evaluating the accumulation and persistence of the Cry1Ac (synpro) and Cry1F (synpro) proteins in several different soils in various strata.

4) Additional empirical data or published literature (other than Gould et al., 2002) that supports the use of CBW alternate hosts as natural refugia. Data would include larval and adult production of CBW on each alternate host for each generation relative to cotton and WideStrike® cotton and the spatial scale and source of moth production.

5) A revised CBW resistance management model based on the recommendations of the June 8-10,

2004 SAP report dated August 19, 2004 should Dow AgroSciences choose to amend the WideStrike® cotton insect resistance management plan.

As discussed above, the registration applicant has submitted or cited data sufficient for EPA to determine that a conditional registration under FIFRA 3(c)(7)(C) for the period ending September 30, 2009, will not result in unreasonable adverse effects on the environment. The human health effects data and non-target organism effects data are considered sufficient for th period of the ocnditional registration. These data demonstrate that no forseeable human health hazards or ecological effects are likely to arise from the use of the product and the risk of resistance developing to the *Bacillus thurigiensis* Cry1F (synpro) and/or *Bacillus thuringiensis* Cry1Ac (synpro) protein(s) during the conditional registration is not expected. The data also demonstrate that, under the terms and conditions of registration (e.g., geographical limitations), there is virtually no possibility of any risk associated with weediness or outcrossing to wild relatives.

Registration of *Bacillus thuringiensis* Cry1F (synpro) and Cry1Ac (synpro) Construct 281/3006 Insecticidal Crystal Proteins as expressed in cotton is in the public interest as required by the second criterion because the benefits (including economic benefits) from the use of the new active ingredient meets or exceeds those of alternative registered pesticides and other available non-chemical techniques. In addtition, EPA believes that the new plant-incorporated protectants are comparatively less risky to health of the environment than currently registered chemical pesticides and provide additional benefits for iinsect resistance management to the currently registered *Bt* cotton plant-incorporated protectants.

In view of the minimal risks and the clear benefits related to WideStrike® cotton, EPA believes that the use of the product during the limited period of the conditional registration will not cause any unreasonable adverse effects.

Although the data with respect to this particular new active ingredient are satisfactory, they are not sufficient to support an unconditional registration under FIFRA 3(c)(5). Additional data are necessary to evaluate the risk posed by the continued use of this product. Consequently, EPA is imposing the data requirements specified earlier in this document in Section III.

EPA has determined, as explained in the Section II.F., that the third criterion for a FIFRA 3(c)(7)(C) conditional registration has been fulfilled because the use of *Bacillus thuringiensis* Cry1F (synpro) and Cry1Ac (synpro) Construct 281/3006 cotton under this registration would be in the public interest.

Conclusion

The submitted data in support of this registration under 3(c)(7)(C) of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) have been reviewed and determined to be adequate. Studies mentioned above are included in the terms, conditions, and limitations of this registration. EPA determines that, for the period of the conditional registration, this registration will not cause unreasonable adverse effects to man or the environment and is in the public interest.

Based on the data submitted and cited by the applicant and reviewed by the Biopesticides and Pollution Prevention Division, EPA has concluded that Dow AgroSciences' Cry1F(synpro)/Cry1Ac (synpro cotton product (WideStrike® cotton) containing the new active ingredients *Bacillus thuringiensis* Cry1F (synpro) and Cry1Ac (synpro) proteins and the genetic material necessary for their production in Event 281-24-236/3006-210-23 cotton be REGISTERED under FIFRA section 3(c)(7)(C), with appropriate limitations.

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Appendix 1

Remedial Action Plan for Responding to Pink Bollworm Action Plan for Pink Bollworm (September 29, 2001)

I. Definitions

Definition #1. Putative Resistance Event--A Cautionary Alert
A putative resistance event consists of any field of *Bt* cotton in which collections of 100 bolls yield =3% large larvae (>3rd instar), pupae or PBW exit holes in bolls. This is a cautionary alert and must not be construed to be a verified resistance event until: 1) the plants from which collections were made are confirmed to produce *Bt* toxin and, 2) bioassays are completed that confirm the reduced susceptibility of the pink bollworm surviving on *Bt* cotton.

Definition #2. A Verified Resistance Event.
A putative resistance event becomes verified if three conditions are met:
A sample of 1000 bolls yields =3% containing large larvae (=3rd instar), pupae, or PBW exit holes.

An ELISA test for *Bt* toxin yields a positive response for *Bt* toxin in a sample of 25 young bolls collected from plants on which PBW larvae were found in the cotton field of interest.
Standardized laboratory bioassays demonstrate that the PBW population of interest is significantly less susceptible to Cry1A(c) toxin than were baseline populations in 1997 (Simmons et al. 1998 and unpublished).

II. Remedial Action

Putative Resistance Event: Year of First Detection.
Within one week of confirming that a Bt field has =3% of bolls containing large larvae (>3rd instar), pupae, or PBW exit holes, alternative PBW controls should be implemented in that field. Measures should include one or more of the following:

- Adulticide treatments if crop is in active growing state, followed by additional insecticide applications (2) on a 3-day schedule, or based on adult emergence as predicted by phenological models.
- If crop is senescent, consider chemical termination to reduce squares and bolls less than 10 days old, accelerate harvest, and destroy crop residue by shredding of stalks followed by discing, and deep plowing (6" burial).
- If crop is defoliated, accelerate harvest and destruction of crop residue to further limit survival of resistant pink bollworm. Destroy crop residue as indicated above.

Verified Resistance Event: Year of First Detection.
If resistance is verified in time to permit it, we strongly recommend that measures be taken to reduce the numbers of resistant pink bollworm that survive to the next season. These could include: adulticide treatments, early termination, and early plowdown, consisting of shredding of stalks followed by discing, and deep plowing (6" burial). Winter irrigation is also recommended to reduce survivorship of overwintering larvae.

Bt fields in the immediate vicinity of a verified resistance event should be examined to detect unusual survivorship of PBW. Results should be used to delimit the size of the affected area and to define the '*Bt* remedial action zone.' We suggest sampling 300 bolls from all *Bt* fields located within the 8 sections of land (designated by © in the adjacent figure) that surround the section of land on which the verified event (VE) occurred. *Bt* cotton fields containing =3% bolls infested with PBW should be considered affected by resistance for the purpose of delimiting the remedial action zone.

The '*Bt* remedial action zone' should be delineated using GPS mapping technology currently in use at the ACRPC. This will ensure accurate records of locations of verified resistance. The remedial action zone should include all sections of land falling within 6 miles of the perimeter of the section(s) of land in which verified resistance events occurred (see figure below).
At such time as fields with verified resistance are detected in >3 different townships within a particular cotton growing region, the entire region may be designated as a *Bt* resistance remedial action zone.

Verified Resistance Event: Next Year's Actions.
Only non-*Bt* cotton should be planted in the remedial action zone in the year(s) immediately following verification of resistance. This measure should be maintained until such time as bioassays of PBW from the remedial action zone demonstrate that the frequency of resistant individuals has declined to acceptable levels. What will constitute levels of resistance acceptable for allowing resumption of use of *Bt* cotton will be determined on an *ad hoc* basis by our Working Group, based on research experience that members have obtained from studies of pink bollworm resistance to Cry1Ac.

The ecological fitness of PBW resistant to Cry1Ac is not known at this time and the dynamics of resistance in the field will likely be influenced by factors including overwintering survival of resistant larvae, intensity of resistance to Cry1Ac, and growth and survival of resistant PBW on *Bt* and non-*Bt* plants. Therefore, new information derived from field and laboratory studies currently underway will be pivotal for determining at what frequency of resistance to Cry1Ac could use of *Bt* cotton expressing Cry1Ac reasonably be resumed within an area previously designated as a *Bt* remedial action zone.

It is assumed that published University recommendations for monitoring and chemical control of pink bollworm will be followed within remedial action zones in order to limit survival of resistant pink bollworm. Additionally, timely crop termination (no top-crop) and early and thorough crop destruction, as detailed above, is strongly encouraged. Releases of sterile pink bollworm and parasitic nematode treatments should also be considered.

The recommendations of our working group regarding 1) *Bt* refuge management and 2) remedial action for responding to PBW resistance in Arizona should be re-evaluated annually and modified to account for new findings. Educational programs and regulatory measures should be devised to promote a high level of producer compliance with recommendations.

III. Organizational Roles

The Arizona Department of Agriculture should serve a central role in implementing this plan, compiling statistics on use of *Bt* cotton, and promoting compliance with remedial action. Consideration should be given on a case-by-case basis for making funds available to compensate producers for costs associated with implementing the remedial action measures recommended herein.

A sampling team comprising personnel from relevant organizations (ACRPC, UA, USDA) will be formed. This team will be ready in August of every year to conduct the sampling required to delineate resistance problems (as detailed above). Similarly, facilities and personnel at EARML will be prepared to conduct bioassays of up to 40 different populations of PBW per season. Funding for these efforts must be sustained.

The registrant should agree to suspend Bt cotton sales in remedial action zones until such time as either the frequency of resistant individuals is shown to have declined to levels deemed acceptable by our Working Group, or new Bt products free of cross-resistance are introduced, and the Arizona Bt Cotton Working Group has concluded that a modified resistance management strategy has been adopted that will adequately reduce the rate of development of further resistance to Bt cotton products.

Appendix 2

Interim Remedial Action Plan for Cotton Bollworm and Tobacco Budworm (September 29, 2001)

1. Actions required for "suspected" resistance events (YEAR 1)

The registrant must instruct its customers (growers and seed distributors) to contact them (e.g., via a toll-free customer service number) if incidents of unexpected levels of tobacco budworm and/or cotton bollworm damage occur.

If the registrant confirms that the level of damage is atypical for *Bt* cotton, the registrant must investigate any field performance issues and determine if the cause of the field performance is:

a. Incorrect insect pest identification.
b. Non-*Bt* cotton or mixed seed in the field. Plant tissue will be collected and sent to the registrant for toxin expression studies (including immunoassays to determine quantitative expression levels of Cry1Ac protein).
c. Low expression of the toxin by the plant, determined by assays described in b. above.

Upon the registrant's confirmation of plant expression of Cry1Ac at expected levels in cotton tissue in at least 98% of the *Bt* cotton plants, and confirmation that the pest is a target pest, laboratory bioassays and genetic methods will be used to determine whether the collected tobacco budworm or cotton bollworm population exhibits a resistant phenotype or genotype. Larvae will be collected by the registrant and delivered to USDA/ARS for diagnostic dose, dose mortality, and allelic recovery tests for the F1, F2, (or F3 if needed) generations.

The registrant must instruct growers to use appropriate alternate control measures on the *Bt* cotton fields to control the potentially resistant pest populations in the subject field only.
The registrant will work with local consultants or state entomologists to monitor the subject field for the remainder of the season or until resistance are confirmed NOT to be the cause. The registrant will instruct growers to use alternate control measures based on the results of the discriminating dose bioassay indicating tolerance/resistance.

The response actions, except for the follow-up monitoring, will be initiated within three days of notification of a problem field. The registrant may solicit the assistance of local state research or extension entomologists and or cotton consultant(s) for the collection and subsequent field monitoring.

2. Actions required to confirm resistance event. (YEAR 1)

If the plant tissue assays (in #1) confirm that toxin expression is adequate AND diagnostic/discriminating dose or dose mortality indicate an increase in tolerance/resistance in problem field(s), conduct diagnostic/discriminating dose, dose mortality, or F2 studies on problem field(s) collection AND collect adults/larvae from surrounding non-*Bt* cotton fields to confirm resistance from the field and in the surrounding general population.

Collections will be made using personnel including the registrant, USDA, state entomologists and cotton consultants.

Dose mortality, diagnostic/discriminating dose, allelic recovery, and other confirmatory tests (as described below) will be conducted with the collected larvae from fields in which resistant individuals may be present by the registrant and USDA/ARS/ and interested parties.
The following definition of resistance will be used for TBW and CBW:
A resistance event becomes verified if the progeny of the sampled TBW and/or CBW population exhibit the following characteristics in bioassays initiated with neonates:

i) if there is > 5-10% survival and > 25% leaf area damaged in a 5-day bioassay using Cry1Ac-positive leaf tissue under controlled laboratory conditions. Note: Since there is not a high dose for CBW, this assay only applies to TBW

ii) if standardized laboratory bioassays using diagnostic doses for TBW and CBW (as currently used by USDA/ARS/) demonstrate resistance has a genetic basis and confirmed survivorship in excess of 1% in a random population sample.

iii) if an LC_{50} in a standard Cry1Ac diet bioassay exceeds the upper limit of the 95% confidence interval of the standard unselected laboratory population LC_{50} for susceptible CBW and TBW populations, as established by the ongoing baseline monitoring program.

If resistance is confirmed, studies must be undertaken to determine the mechanism of resistance (e.g. binding site modifications, etc.).

Once resistance is confirmed, the registrant must notify EPA within 30 days and work with the Agency to establish a resistance mitigation plan. Concurrently, surrounding *Bt* cotton fields will be monitored by the registrant and cotton consultants/state entomologists throughout the season for unusually high survival incidences of bollworm or tobacco budworm. Populations with unusually high survival will be collected and tested in the discriminating dose assay. The registrant will instruct growers to arrange for early harvest of the crop and fall or spring plowing to reduce the potential for overwintering pupae.

Once all data are available from the dosage-mortality, diagnostic dose, and/or F2 tests and field monitoring studies and resistance is confirmed, the registrant will convene a meeting prior to the subsequent season with the local state entomologists, cotton consultants, USDA personnel, seed companies, Federal (EPA) and State regulatory officials, and invited experts to determine the most appropriate course of action in the next season, relative to the pest involved and the area affected. This group will also develop a plan for more intensive monitoring and a revised insect resistance management plan in the affected county(s) to determine if the resistance can be detected in the subsequent year, the level of resistance, and the prevalence of the resistance.

3. Mitigation strategies required for YEAR 2 and beyond

Unless modified by EPA pursuant to the consultation process described below, the registrant will

suspend sales of *Bt* cotton in the affected area (e.g. county in which the resistance event occurred) and take the steps below until resistance allele frequencies have been demonstrated to have returned to acceptable levels (as defined by a group of experts). At the request of the registrant, EPA will consult with the registrant and academic experts concerning possible alternative resistance mitigation. If EPA determines such strategies would be sufficient to contain confirmed resistance, EPA may approve the use of such strategies instead of the measures specified in this interim plan.

The registrant will inform growers, state entomologists, consultants, seed companies and distributors/dealers in the county and adjacent counties of the affected area of the confirmed resistance event prior to the start of the growing season. This communication will also include the following:

- intensification of field monitoring of damage/insect infestations
- timely and appropriate use of insecticide alternate control measures
- early crop harvest, early stalk destruction avoiding regrowth, fall/spring plowing to destroy overwintering pupae

The registrant will increase resistance monitoring in all affected areas and adjacent counties or other areas in which resistance may be likely. More specific recommendations may be developed through winter meetings by the registrant and local cotton experts that are tailored to the specific pest/situation.

Once resistance is confirmed for the defined areas, the registrant will convene a meeting annually with local, state, industry, EPA, and USDA experts to refine mitigation strategies for the following growing season specifically tailored to the pest/situation. Refinements could include the following elements:

a. Alternate control measures
b. Reduce/eliminate *Bt* cotton use
c. Reduce/eliminate cotton use
d. Modify refuge requirements
e. Lab generated susceptible male release (sterile if appropriate)
f. Spring/early summer trap crops with alternate control measures
g. Area wide virus treatments over spring hosts
h. Continue field and resistance monitoring in expanded areas
i. Use feeding or mating attractants in *Bt* fields to improve susceptible male (or female if using feeding attractants) movement and random-mating with resistant insects

Each of the above strategies must be carefully considered based on the existing knowledge of:

a. Extent/severity of the resistance problem
b. Agreed upon value/potential success of the tactic (including use of reversion models)
c. Practicality/feasibility of the tactic
d. Cost/availability of alternate control technologies
e. Value of the crop or technology to the grower
f. Availability of funding sources