USDA

United States Department of Agriculture

Animal and Plant Health Inspection Service

Marketing & Regulatory Programs Business Services

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Riverdale, MD
20737

JUL 16 2004

Dr. Irene Gatti
Regulatory Leader – Biotech
Dow AgroSciences LLC
9330 Zionsville Road
Indianapolis, IN 46268

Dear Dr. Gatti:

Your petitions numbered 03-036-01p and 03-036-02p for determinations of nonregulated status for cotton transformation events 281-24-236 and 3006-210-23, respectively, were approved on July 15, 2004. Enclosed are signed copies of the Environmental Assessment (EA) and Finding of No Significant Impact, with the attached summary and response to comments on the EA and petitions, and the determination statement, all of which cover both petitions. These documents will also be posted soon on the BRS website at http://www.aphis.usda.gov/brs/ea_pubs.html. Should you have any questions about these documents, please contact Ms. Terry Hampton, Secretary of the BRS Regulatory Division, at Area Code (301) 734-5715. Ms. Kay Peterson is no longer handling petition related documents as she has accepted a new position within APHIS.

The Animal and Plant Health Inspection Service must be notified within five days in writing if any information comes to the applicant's attention that differs substantially from what was described in the petitions and our environmental analysis.

A notice advising the public of our determination that the transformation events and their progeny are no longer considered regulated articles under 7 CFR 340 has been prepared for publication in the *Federal Register*. You will be advised of the publication date when it becomes available.

Sincerely,

Cindy Smith
Deputy Administrator
Biotechnology Regulatory Services

Approval of Mycogen/Dow Petitions 03-036-01p and 03-036-02p
Seeking Determinations of Nonregulated Status for Insect-Resistant Cotton Events
281-24-236 and 3006-210-23 Genetically Engineered to Express Synthetic
B.t. Cry1F and Cry1Ac, Respectively

Environmental Assessment and
Finding of No Significant Impact

July 2004

The Animal and Plant Health Inspection Service (APHIS), United States Department of Agriculture (USDA) has prepared an environmental assessment (EA) prior to approving petitions (03-036-01p and 03-036-02p) for determinations of nonregulated status received from Mycogen Seeds c/o Dow AgroSciences LLC (Mycogen/Dow) under APHIS regulations at 7 CFR Part 340. The subject of these petitions, cotton transformation event 281-24-236 and cotton transformation event 3006-210-23 are genetically engineered to express synthetic insecticidal proteins Cry1F and Cry1Ac, respectively, for resistance to certain lepidopteran insect pests, and are also engineered to express the enzyme phosphinothricin acetyltransferase (PAT) as a selectable marker for transformation that confers tolerance to the herbicide glufosinate-ammonium. On March 9, 2004, APHIS published a notice in the Federal Register (69 FR 10972-10973, Docket no. 04-010-1) announcing the availability of the petition and EA for public review and comment. During the designated 60 day comment period, APHIS received 6 comments. APHIS' analysis of and response to these comments is included as an attachment to this finding. Based on the analysis in the EA and in our response to the comments, APHIS has reached a finding of no significant impact (FONSI) to the environment from its determination that the subject transformation events and progeny derived from them, including progeny derived from crossing these events, shall no longer be considered regulated articles. This determination is attached to the EA as Appendix E.

Neil C. Hoffman for Cindy Smith

Cindy Smith
Deputy Administrator
Biotechnology Regulatory Services
Animal and Plant Health Inspection Service
U.S. Department of Agriculture
Date JUL 15 2004

APHIS' Analysis and Response to Comments Received on Petitions 03-036-01p and 03-036-02p and the EA.

APHIS received 6 comments on the petition and the EA during the 60-day comment period. The comments were received from individuals, cotton growers, academic researchers, and an organization representing the US cotton industry. The comments in favor of determinations of nonregulated status for the two subject cotton transformation events totaled 5 (with one of those requesting partial deregulation for both events) and one comment opposed the deregulations. Comments in favor of deregulation stressed benefits to the environment, cotton growers, and society; greater efficacy in managing lepidopteran pests of cotton and insect resistance afforded by the availability of cotton with different stacked Bt toxins that could result from crosses between the subject cotton transformation events; and greater competition in the development and marketing of enhanced cotton lines in different varieties suitable for a variety of growing regions. It was noted that a continuation in the reduction in insecticide use in cotton is expected as for previously deregulated insect-resistant cotton transformation events under commercial production.

The comment in opposition to deregulation for the subject cotton events was generally not germane to the specific cotton events in question or any specific hazard that they posed. The comment suggested that more field testing is required for the subject cotton lines, and that APHIS and FDA regulations and policies on products of biotechnology need to be updated. APHIS is currently in the process of examining our regulations, and has published in the Federal Register (69 FR 13280-13281) a Notice of Intent to prepare an environmental impact statement and seek public comment on this process. The current regulations at 7 CFR 340 apply to the process of making a determination of nonregulated status for the subject cotton events until such time that a revised final rule is promulgated. The subject petitions were deemed to be technically complete and contained sufficient field test data in accordance with APHIS guidelines for preparing and submitting a petition (available at the APHIS, BRS website http://www.aphis.usda.gov/brs/index.html).

The commenters in favor of partial deregulation for the subject cotton events raised the following concerns, which are addressed below.

1. **Geographical limitations should be considered to address the concern that cross-pollination could occur to wild cotton species in Southern Florida, Hawaii and Southern Arizona.**

As noted in the Biopesticides Registration Action Document of 2001 referenced in the EA, an October 2000 Scientific Advisory Panel (SAP) meeting supported EPA's regulatory decisions to prohibit commercial Bt Cry1Ac cotton production in southern Florida and Hawaii where wild (or feral) cotton plants are known to exist. The concern cited was the development of weediness, but also concerns of biodiversity and loss of genes that might provide value in plant breeding. EPA cites the lack of basic biological data (e.g., pollinator

ecology, compatibility/sterility factors, potential impact of Bt on herbivores, distribution of native populations) on *G. tomentosum*, the wild Hawaiian cotton, as justification for measures to mitigate hybridization with cultivated cotton on these islands.

APHIS analyzed the potential for cross-pollination to occur with wild cotton and related species in the United States in the EA, and in Appendix A, and concluded that it is very unlikely that Cotton Events Cry1F and Cry1Ac will successfully cross with wild sexually compatible relatives when grown in the United States and that if this does occur, the offspring are not likely to pose an increased risk of weediness. APHIS also concluded that no impacts are expected on species listed as threatened or endangered. Successful cross-pollination of *G. hirsutum* cotton with wild *G. thurberi* in southern Arizona is unlikely due to chromosomal differences. Furthermore, wild populations of cotton in Florida and of *G. tomentosum* in Hawaii are isolated from areas of commercial cotton production. Commercial production of cotton does not occur in Hawaii, although a limited amount of cotton seed production does occur there (less than 204 acres in 2003-2004 according to National Agricultural Statistics Service Data available at http://www.nass.usda.gov/hi/speccrop/seed.htm).

A query of a database maintained by the Smithsonian Institution on flora of the Hawaiian islands on June 2, 2004 (available at http://ravenel.si.edu/botany/pacificislandbiodiversity/hawaiianflora/query2.cfm), indicated that *G. tomentosum* is native to seven of the eight Hawaiian islands (Niihau, Kauai, Oahu, Molakai, Lanai, Maui, and Kahoolawe), based on the Manual of Flowering Plants published by W.L. Wagner, S.H. Sohmer in 1990. The database indicates that this species has no official special protected status in the United States, but is assessed as a vulnerable population through the collective efforts of personnel of the Bishop Museum, The Nature Conversancy, the Smithsonian Institute, and the U.S. Fish and Wildlife Service. Taxa listed as vulnerable are defined as "likely to become endangered in the near future unless the threats to their survival are removed or reduced. In the Hawaiian Islands, most species in this category are threatened by extensive habitat destruction or modification or by other environmental disturbances."

Recent preliminary results of EPA funded research by Drs. John Pleasants and Jonathon Wendel of Iowa State University on *G. tomentosum* populations in Hawaii provide new information about their distribution, the timing of flowering, and potential pollinators (Memorandum dated April 8, 2004 to Janet Andersen, Director, Biopesticides and Pollution Prevention Division (BPPD), USEPA, from Tessa Milofsky, Regulatory Action Leader, BPPD, documenting a conversation with Jonathon Wendell regarding their research; and personal communication from Dr. Pleasant to Susan Koehler, USDA, APHIS, June 1, 2004). Natural populations are found on all the islands except Kauai (in contrast to historical records) and Hawaii. The species is dominant on the Hawaiian island of Kohoolawe and several sizable populations were found on the islands of Oahu and Maui. The sparse populations observed on Molokai appear to be threatened by recent ecological alterations, resulting from farming and ranching activites that have decimated much of the island's native flora. In some places *G. tomentosum* has been planted for habitat restoration or roadside or stream bank stabilization. Dr. Pleasants indicated

that there was no evidence that it is being controlled as a weed in any of the habitats that they have observed. While the plants are primarily self-pollinating, in contrast to earlier reports that the flowers open at night and may be cross-pollinated by a moth, their research found that the flowers appear to open at sunrise, and pollen is viable until about 4-5 pm, corresponding with the pollination window for *G. hirsutum*. Furthermore, hymenopteran insects, including the honey bee, carpenter bee, and an unidentified small black bee, were observed as frequent visitors and possible pollinators in *G. tomentosum.* Although bees are capable of transporting pollen long distances (up to 12 km from their hive), the researchers noted that the homogeneity of the *G. tomentosum* populations suggests that insect mediated pollination events are infrequent between distant populations.

It is unclear how gene introgression from Bt lepidopteran resistant or glufosinate tolerant cotton lines would cause a loss in genes valuable to plant breeding any more so than would gene flow from other improved cotton varieties. The USDA, ARS, National Genetic Resources Program, *Germplasm Resources Information Network - (GRIN),* online database, maintained by the National Germplasm Resources Laboratory, Beltsville, Maryland, when accessed on June 2, 2004, listed 29 accessions of *G. tomentosum* of which 16 were currently available for distribution (Available at: http://www.ars-grin.gov/cgi-bin/npgs/html/tax_stat.pl?17948). These collections are maintained by Dr. A. E. Percival at the USDA, ARS, Crop Germplasm Research Unit, in College Station, Texas. APHIS (Susan Koehler) personally contacted Dr. Percival and other researchers at this location on June 1-2, 2004 to determine the significance of this germplasm as a source of insect resistance genes. Dr. Percival indicated that he could not recall any seed request from cotton breeders working to develop insect resistant lines of any kind using *G. tomentosum*. He noted that this species has glands and short leaf hairs that may attract some species and deter others, and that another researcher, Dr. David Stelly, Texas A&M said that he has observed some resistance to bollworm feeding in some of their research plots, but this work is not published. Dr. John Westbrook, a USDA, ARS scientists with expertise in cotton pest ecology, also questioned other scientists at the facility, including Dr. Juan Lopez, a bollworm expert, and indicated that they are unaware of any information on resistance of *G. tomentosum* to bollworms or any other cotton insect pests. Thus it appears that *G. tomentosum* is not recognized as an important germplasm source for insect resistance genes. Although introgression to *G. tomentosum* of transgenes conferring lepidoperan resistance or glufosinate tolerance from cotton, including the lines considered for deregulation, is unlikely to occur at a high frequency in the absence of mitigation measures, if it were to occur, it would likely confer some resistance to the lepidopteran pests targeted by the Bt toxins and some tolerance to glufosinate ammonium herbicides. It is unclear how important these pests or herbicides might be in limiting the existing populations of *G. tomentosum* in the Hawaiian islands in which they have been found. If they are a source of significant selection pressure, then transgene flow could potentially help stabilize these populations. It did not appear to Dr. Pleasants that *G. tomentosum* was being actively controlled as a weed.

APHIS has not identified a significant impact to the wild cotton in southern Florida or Arizona or to *G. tomentosum* in the Hawaiian islands that would arise from gene introgression from the subject transgenic cotton lines that warrant APHIS placing geographic restrictions on their determination of nonregulated status. APHIS notes that EPA is likely to consider new information resulting from the efforts of Drs. Pleasants and Wendel in deciding whether to place geographical restrictions on these plant-incorporated-protectants as part of their registration as they have for other Bt cottons, to address the potential concerns raised by the SAP.

2. **Maintenance of suitable refuges between commercial B.t. cotton and organic cotton fields should be monitored.**

It was unclear from the comments whether this refuge was being proposed to prevent gene flow to organic cotton production fields or to delay the development of resistance to the B.t. toxins expressed in the cotton events. Insect resistance management (IRM) is addressed in the points below. APHIS' response to comments on the Bollgard II cotton petition and EA on the issue of concerns to organic farmers applies equally in response to the comment raised here. APHIS notes that a determination of nonregulated status for the subject cotton events under 7 CFR Part 340 does not affect the provisions of the National Organic Program (NOP) administered by USDA's Agricultural Marketing Service. The NOP considers that the presence of a detectable residue alone does not necessarily indicate use of a product of excluded methods that would constitute a violation of the standards. (Please refer to the preamble of the NOP final rule at residue testing, changes requested but not made, (3) Threshold for Genetic Contamination for a discussion of "adventitious presence" in relation to organic production; available on-line at website: http://www.ams.usda.gov/nop/nop2000/Final%20Rule/preamble/pre-residues.htm.) In addition, the NOP requires that organic production operations have distinct, defined boundaries and buffer zones to prevent unintended contact with prohibited substances from adjoining land that is not under organic management. The organic system plan enables the production operation to achieve and document compliance with the National Organic Standards, including the prohibition of the use of excluded methods.

3. **Further field test data on stacked Bt toxin cotton lines (both those derived from the subject cotton events and Bollgard II, which contains Cry1Ac and Cry2Ab toxins, should be evaluated prior to deregulation for the stacked hybrid cotton Cry1Ac/Cry1F line (MXB-13) in order to better predict ecological effects.**

APHIS notes in the EA that the subject Cotton Events Cry1F and Cry1Ac have been field tested in a variety of locations in 13 states that represent major cotton growing regions, and that the stacked MXB-13 cotton line was also field tested in these states in 2001 and 2002 in the same locations as the individual cotton transformation events under consideration. Furthermore, a two year, two location field study submitted by Mycogen/Dow did not detect any consistent major negative effects on non-target arthropod abundance in the stacked cotton treated with recommended insecticides to control non-lepidopteran pests as compared to non-transgenic cotton similarly treated or treated with conventional insecticides. APHIS' EA for the

deregulation of Bollgard II noted that the petition included extensive information on the attributes of stacked Cry1Ac/Cry2Ab cotton event 15985 gathered from field tests conducted during 1998 and 1999 in 98 field test locations in the major cotton producing areas of the US and in over 250 field trials conducted in the US, Puerto Rico, Argentina, South Africa, and Australia. In both EAs, toxicity data from laboratory studies also support the conclusion that significant impacts on non-target organisms is not expected. The actual impacts of these stacked Bt. cotton lines on IRM will not be known for several years, but IRM monitoring has been included as a condition of the pesticide registrations for other Bt cotton. Thus far resistance to Bt toxins as a result of the deployment of transgenic corn or cotton, including those expressing Cry1Ac and Cry1F, has not developed and stacked (or pyramided) Bt toxin products, particularly toxins with different or unshared target binding sites, are expected to delay development of resistance (Sharlene Matten, EPA, personal communication to Susan Koehler, May 28, 2004). Information on Bollgard II cotton was presented at the January 2004 Beltwide Cotton Conference held in San Antonio, Texas. Based on all research and reports presented at the Conference, it can be concluded that there are no significant adverse non-target or ecological effects from planting Bollgard II cotton. On the contrary, it was reported that Bollgard II led to increased productivity compared to non-Bt cotton. Benefits from Bollgard II (Cry1Ac/Cry2Ab) cotton are expected to outweigh those resulting from planting Bollgard (Cry1Ac) cotton.

The EPA is convening a FIFRA SAP open meeting on June 8-10, 2004 (see FIFRA SAP web site **http://www.epa.gov/scipoly/sap/)** to address product characterization, human health risk, ecological risk, and insect resistance management for Bt cotton products. In their announcement, EPA reaches the following conclusions in their assessment of ecological risk and insect resistance management for the stacked Cry1Ac/Cry1F cotton (WideStrike™):

1) no synergistic effects or increase in non-target host range were seen as a result of combining these two proteins in the same product,
2) aquatic and terrestrial wildlife were not likely to be harmed and WideStrike cotton was not likely to threaten the long-term survival of any non-target wildlife populations,
3) the Agency has sufficient information to conclude that there is no hazard from the proposed uses of WideStrike cotton to non-target wildlife, aquatic and soil organisms, but they are requesting additional, primarily long term effects data that were recommended by previous Panels for PIP corn to lend additional weight to their conclusion.
4) 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.

APHIS is sufficiently certain that based on the data analyzed and impacts of the commercial use of other Bt cotton varieties that no significant ecological impacts are expected, and that should they arise, they could be detected and managed based on the conditions placed on such products for pesticide registration by the EPA.

4. Although under the authority of the EPA, APHIS should stipulate that an Insect Resistance Monitoring Program is put in place and enforced as part of the deregulation decision.

APHIS' analysis of cumulative impacts support a FONSI in the absence of the proposed conditional deregulation. APHIS notes that an IRM plan has been developed for Widestrike cotton and is being considered at the SAP meeting discussed above. Included in components of the plan are the use of refuges, annual resistance monitoring, an annual compliance assurance program, grower education, remedial action plans, and annual reporting. Past experience with IRM implementation for Bt crops as stipulated in conditions to pesticide registrations, and the availability of a number different Bt cotton varieties, support the conclusion that additional measures on the part of APHIS are unnecessary to address the issue of IRM. Furthermore, APHIS does not have the authority to compel or enforce compliance with regulations of another agency.

These commenters also suggested that APHIS should more thoroughly investigate:

1) the allergenicity potential of the B.t. toxins and PAT proteins expressed in these cotton events,
2) potential for impacts resulting from overexpression of the PAT proteins,
3) international applications of the cottons lines, and
4) effects on non-target arthropods.

These points are addressed below.

Allergenicity of the pesticide active ingredients is assessed by the EPA during product registration. APHIS' EA (Appendix C and D) did acknowledge that studies were submitted to both APHIS and the EPA indicating that Cry1F (synpro), Cry1Ac (synpro) and PAT do not exhibit characteristics commonly attributed to allergenic proteins. The allergenic potential of Cry1F, Cry1Ac and PAT proteins have also been the subject of previous APHIS and EPA risk assessments and are expressed in other products that have been commercialized, and APHIS is unaware of any reports of allergenicity resulting from these products.

PAT protein expression levels in the lines considered for deregulation are relatively low, especially in the Cry1Ac cotton event. For example, levels measured in the seeds (based on data submitted in both of the petitions) if combined would average 0.53 μg/g fresh weight, and this is 254 times lower that the highest average value measured (135 μg/g fresh weight) in cleaned cotton seed from a previously deregulated glufosinate tolerant line of cotton (see petition 02-042-01p, pg. 42). In as much as the PAT protein is not known to have toxic effects (for example see FDA's analysis of PAT protein safety for the food safety consultations for BNF numbers 55, 23, 29, and 46 available at http://vm.cfsan.fda.gov/%7Elrd/biocon.html), over-expression of this protein, should it occur, is unlikely to have an adverse effect on wildlife or humans, but may confer greater tolerance to the herbicide glufosinate ammonium.

APHIS regulations at 7 CFR 340 do not cover the export of regulated articles. Therefore export of the subject cotton lines is not effected by a decision to deregulate. APHIS did address the potential environmental impacts outside the United States associated with a decision to deregulate in the EA (see pp. 24-25). As noted, the decision of a foreign country to allow importation or use of the subject cotton lines may be regulated by national biosafety legislation and may be subject to phytosanitary regulations or measures. The commenters suggested that events in Madhya Pradesh, India warrant caution in commercialization of these cotton lines. According to an article in an Indian magazine, Frontline (Vol. 21- Issue 10, May 08-24, 2004), India's Genetic Engineering Approval Committee has approved four Bt cotton seed varieties containing the Cry1Ac gene developed by Monsanto to be released for commercial cultivation, three of which were approved in March 2002. The article reports that in the first year of commercial cultivation of the first three lines approved, failed or unsatisfactory harvests were obtained from different parts of the country, including in Madhya Pradesh where non-Bt plants performed much better. APHIS notes that the regulatory authority authorizing release or commercialization of a bioengineered crop should evaluate sufficient field data to determine, according to their regulatory authority, the environmental impacts and agronomic performance associated with cultivation of the crop. The performance of these varieties in India may not be predictive of impacts of the cotton events under current consideration by APHIS, as these events are introduced in different genetic backgrounds and are intended to be commercialized as a stacked Bt product.

APHIS did analyze the potential effects on non-target arthropods on pp. 16-21 of the EA. This included analysis of data on the specificity of the Bt proteins, toxicity data on beneficial arthropods, as well as field study data. APHIS does not feel that further information is warranted to conclude that no significant non-target effects are expected from a deregulation of the subject cotton events.

USDA-APHIS Decisions on Mycogen/Dow Petitions 03-036-01p and 03-036-02p Seeking Determinations of Nonregulated Status for Insect-Resistant Cotton Events 281-24-236 and 3006-210-23 Genetically Engineered to Express Synthetic B.t. Cry1F and Cry1Ac, Respectively

Environmental Assessment

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I. SUMMARY

The Animal and Plant Health Inspection Service (APHIS) of the United States Department of Agriculture (USDA) has prepared an Environmental Assessment (EA) prior to making determinations on the regulated status of cotton (*Gossypium hirsutum* L.) lines designated as B.t. Cry1F synthetic protoxin (synpro) cotton event 281-24-236 (hereafter referred to as Cotton Event Cry1F) and B.t. Cry1Ac cotton event 3006-210-23 (hereafter referred to as Cotton Event Cry1Ac). Cotton Event Cry1F and Cotton Event Cry1Ac have been genetically engineered to express different synthetic bacterial genes from *Bacillus thuringiensis* (B.t.) which enable the cotton lines to resist feeding damage from lepidopteran insects.

APHIS has prepared this EA in response to two separate petitions received from Mycogen Seeds c/o Dow Agrosciences LLC (hereafter referred to as Mycogen/Dow) for determinations by APHIS that Cotton Event Cry1F and Cotton Event Cry1Ac do not present a plant pest risk, and therefore should no longer be considered as regulated articles under APHIS regulations found at 7 CFR Part 340. These petitions (designated 03-036-01p for the Cotton Event Cry1F and 03-036-02p for the Cotton Event Cry1Ac) contain extensive information relevant to making these determinations. It is the stated intention of Mycogen/Dow to commercialize cotton lines derived from Cotton Events Cry1F and Cry1Ac primarily as a stacked product containing both of the synthetic B.t. insecticidal genes. An APHIS determination of nonregulated status for both Cotton Event Cry1F and Cotton Event Cry1Ac would be necessary for this to occur. Cotton Event Cry1F and Cotton Event Cry1Ac have been considered regulated articles under APHIS regulations at 7 CFR Part 340 because some DNA regulatory sequences used to control the expression of the foreign genes in cotton were derived from plant pests, and the vector used to insert the foreign genes is a plant pest.

As a regulated article under the provisions of 7 CFR Part 340, the importation, interstate movement, or cultivation in the environment of these cotton events has been conducted under authorizations from APHIS. These authorizations stipulate conditions of physical and reproductive confinement that preclude the regulated article from becoming mixed with nonregulated articles or persisting in the environment outside the authorized site.

This EA summarizes the APHIS review of potential environmental impacts that might occur from an APHIS determination that Cotton Event Cry1F and/or Cotton Event Cry1Ac should no longer be considered regulated articles under the regulations found at 7 CFR Part 340.

II BACKGROUND

A. Development of Cotton Events Cry1F and Cry1Ac.

Mycogen/Dow developed Cotton Events Cry1F and Cry1Ac primarily so that they can be crossed to produce a commercial line that contains both of the insecticidal proteins which they believe will reduce selection pressure for resistance to insecticides, and help to maintain the range of effective

control options for lepidopteran pests available to cotton growers. Both Cotton Events Cry1F and Cry1Ac also were genetically engineered to express a selectable marker gene for tolerance to the herbicide glufosinate ammonium. This gene was physically linked to the genes encoding Cry1F and Cry1Ac. This enabled the herbicide to be used to select tissue transformed to contain both the insecticidal gene and herbicide tolerance gene. Cotton engineered for tolerance to the herbicide glufosinate ammonium has previously been deregulated by APHIS. The petition 03-036-01p describes field tests in which Cotton Event Cry1F is shown to have very good efficacy against the lepidopteran pests tobacco budworm (*Heliothis virescens*), beet armyworm (*Spodoptera exigua*), and soybean looper (*Psuedoplusia includens*) and moderate levels of efficacy against the cotton bollworm (*Helicoverpa zea*). The petition 03-036-02p describes field tests in which Cotton Event Cry1Ac is shown to provide additional efficacy against most of these pests as well as efficacy against additional pests such as pink bollworm (*Pectinophora gossypiella*) and some additional armyworm and loopers.

Field tests of Cotton Events Cry1F and Cry1Ac have been conducted since 1999 and 2000, respectively in numerous states of the United States and in the territory of Puerto Rico under authorizations granted by APHIS in accordance with regulations at 7 CFR Par 340 (See Appendix B). In most cases data was also obtained on the stacked product (cotton line MXB-13) resulting from crosses between the two Cotton Events Cry1F and Cry1Ac. The stacked product is homozygous for both of the insecticidal protein genes. These tests were conducted in part, to confirm that Cotton Events Cry1F and Cry1Ac exhibit the desired agronomic and quality characteristics and do not pose a greater plant pest risk than the unmodified cotton cultivar from which both of them were derived. APHIS authorizations require that the regulated article not be planted with nonregulated plant material that is not part of the field release, that it be contained or devitalized when no longer in use, and that the regulated article and its offspring must not persist in the environment after completion of the test. Measures were employed during these field tests to achieve physical and reproductive confinement from other sexually compatible plants and to manage volunteer cotton seedlings that arise from germination of seed left on the soil from the previous season's field test.

B. APHIS Regulatory Authority.

APHIS regulations under 7 CFR Part 340, which are promulgated pursuant to authority granted by the Plant Protection Act (Title IV, Pub. L. 106-224, 114 Stat. 438, 7 U.S.C. 7701-7772), regulate the introduction (importation, interstate movement, or release into the environment) of certain genetically engineered organisms and products. A genetically engineered organism is considered a regulated article if the donor organism, recipient organism, vector or vector agent used in engineering the organism belongs to one of the taxa listed in the regulation and is also a plant pest, or if there is reason to believe that it is a plant pest. Cotton Events Cry1F and Cry1Ac have been considered regulated articles because some noncoding DNA regulatory sequences were derived from plant pathogens and the vector used to introduce the foreign DNA is a plant pest.

Section 340.6 of the regulations, entitled "Petition for Determination of Nonregulated Status", provides that a person may petition the Agency to evaluate submitted data and determine that a particular regulated article does not present a plant pest risk and should no longer be regulated. If APHIS determines that the regulated article is unlikely to pose a greater plant pest risk than the unmodified organism from which it is derived, the Agency can grant the petition in whole or in part. Therefore, APHIS permits or notifications would no longer be required for field testing, importation, or interstate movement of that article or its progeny.

C. U.S. Environmental Protection Agency (EPA) and Food and Drug Administration (FDA) Regulatory Authority.

Cotton Events Cry1F and Cry1Ac are also subject to regulation by other agencies. The EPA is responsible for the regulation of pesticides under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136 *et seq.).* FIFRA requires that all pesticides be registered before distribution or sale, unless exempt by EPA regulation. On February 6, 2003, the EPA announced receipt in the Federal Register (68 FR 6147) (FRL-7289-4) of an application from Dow Agrosciences [EPA File Symbol 68467-G] to register the stacked B.t. Cry 1F (synpro)/Cry 1Ac (synpro) insecticidal crystal protein constuct [referred to as 281/3006 in the application] as a plant-incorporated protectant in cotton **(http://www.epa.gov/fedrgstr/EPA-PEST/2003/February/Day-06/p2935.htm**). The EPA has not announced its final decision on this application. Before a product may be registered as a pesticide under FIFRA, it must be shown that when used in accordance with widespread and commonly recognized practices, it will not cause unreasonable adverse effects on the environment.

Under the Federal Food, Drug, and Cosmetic Act (FFDCA) (21 U.S.C. 301 *et seq.),* pesticides added to (or contained in) raw agricultural commodities generally are considered to be unsafe unless a tolerance or exemption from tolerance has been established. Residue tolerances for pesticides are established by the EPA, and the FDA enforces those tolerances. In the Federal Register of October 9, 2002 (67 FR 62971) (FRL-7196-2), EPA issued a notice pursuant to Section 408 of the FFDCA, 21 U.S.C. 346a, as amended by the Food Quality Protection Act (Public Law 104-170) announcing the filing of a pesticide tolerance petition (PP2G494), by Mycogen Seeds, c/o Dow AgroSciences LLC to establish a temporary exemption from the requirement of tolerance for residues of B.t. Cry1F protein and the genetic material necessary for its production in cotton. This genetic material is the same as that introduced into Cotton Event Cry1F that is the subject of the petition 03-036-01p. After analysis of the supporting data and comments received on the petition, EPA subsequently published in the Federal Register (68 FR 23073) on April 30, 2003, its regulation granting this temporary exemption. This temporary exemption expires on May 1, 2004. An Experimental Use Permit [No. 68467-EUP-6] issued to Dow Agrosciences that covers planting of the stacked Cry1F/Cry1Ac construct through April 2004 states that tolerance exemptions listed under 40 CFR 180.1227 and 40 CFR 180.1155 cover the plant-incorporated protectant being tested under this EUP. A request to extend by 1 year both the temporary tolerance exemption for Cry1F in cotton and the EUP have been received by the EPA, but the EPA has not yet announced their decision on this request (Leonard Cole, EPA, BPPD, personal communication with Susan Koehler, APHIS, January 28, 2004). On

July 8, 2003, Mycogen/Dow submitted a request for a full tolerance exemption for both the Cry1F and Cry1Ac proteins as expressed in these Cotton Events Cry1F and Cry1Ac, but the EPA has not yet announced receipt of these requests.

FDA's policy statement concerning regulation of products derived from new plant varieties, including those genetically engineered, was published in the Federal Register on May 29, 1992, and appears at 57 *FR* 22984-23005. Mycogen/Dow submitted summaries of their safety assessments for the Cotton Events Cry1F and Cry1Ac on March 13, 2003, but their food safety and nutritional consultation with the FDA is not yet complete (as of Jan. 28, 2004).

III. PURPOSE AND NEED

In compliance with the National Environmental Policy Act (NEPA) of 1969 (42 U.S.C. 4321 *et seq.)* and the pursuant implementing regulations (40 CFR 1500-1508, 7 CFR Part lb; 7 CFR Part 372), APHIS has prepared this EA before making a determination on the status of Cotton Event Cry1F and Cotton Event Cry1Ac as regulated articles under APHIS regulations found at 7 CFR Part 340.

IV. ALTERNATIVES

It is understood that each of the alternatives listed apply individually to Cotton Event Cry1F and Cotton Event Cry1Ac that are the subject of the two petitions 03-036-01p and 03-036-02p, respectively.

A. No Action: Continuation as a Regulated Article

Under the "no action" alternative, APHIS would not come to a determination that Cotton Event Cry1F and/or Cotton Event Cry1Ac should no longer be considered regulated articles under 7 CFR Part 340. As such, APHIS authorizations would still be required for their introductions, thereby effectively precluding the possible use of the subject Cotton Event and its progeny from typical commercial farming production. APHIS can choose this alternative if there is insufficient evidence to demonstrate lack of plant pest risk from the unconfined cultivation of the subject Cotton Event and its progeny.

B. Proposed Action: Determination of Nonregulated Status, in Whole

Under this alternative, APHIS would determine that Cotton Event Cry1F and/or Cotton Event Cry1Ac or progeny derived from either or both of these would no longer be considered regulated articles under 7 CFR Part 340, because they do not meet the definition described in the regulation. With such a determination of nonregulated status, APHIS authorizations would not be required for introductions of the subject Cotton Event in the United States or its territories. A determination of nonregulated status under 7CFR Part 340 does not preclude any other requirements that might be placed on the use of these plants by other regulations (e.g., registration with EPA).

C. Proposed Action: Determination of Nonregulated Status, in Part

The regulations at 7 CFR Part 340.6 (d) (3) (I) state that APHIS may "approve the petition in whole or in part." There are two ways in which a petition might be approved in part:

1. Approval of some but not all of lines requested in the petition. In some petitions, applicants request deregulation of lines derived from more that one independent transformation event. In these cases, supporting data must be supplied for each line. APHIS could approve certain lines requested in the petition, but not others. Each of the two petitions, which are the subject of this EA, only request deregulation of lines derived from only one transformation event and their progeny.

2. Approval of the petition with geographic restrictions. APHIS could determine that the regulated article poses no significant risk in certain geographic areas, but may pose a significant risk in others. In such a case, APHIS might choose to approve the petition with a geographic limitation stipulating that the approved lines could only be grown without APHIS authorization in certain geographic areas.

V. POTENTIAL ENVIRONMENTAL IMPACTS

APHIS considered potential environmental impacts of each of the three alternatives described in Section IV above.

A. Alternative A: No Action

If APHIS takes no action (i.e., does not grant nonregulated status), commercial scale production of Cotton Event Cry1F and/or Cotton Event Cry1Ac and/or their progeny, including the stacked Cry1F/Cry1Ac cotton line is effectively precluded. These plants could still be grown in field trails for variety development as they have been grown for the past several years, although still under the requirements of APHIS authorizations (permits or notifications) and EPA issued EUPs (if required depending on the use and acreage). APHIS evaluated field data reports submitted by Mycogen/Dow for field trials completed for Cotton Events Cry1F and Cry1Ac and the stacked cotton line at the time the petitions were submitted (see Appendix B of this EA), and comparison of these cotton lines with the non-transgenic control indicated no consistent significant adverse effects on non-target arthropods, no increase in weediness characteristics, and no effect on the health of other plants. The Agency expects that future field tests under APHIS authorizations would be similar.

With respect to commercial production, APHIS believes that without the option of cultivating Cotton Event Cry1F, Cotton Event Cry1Ac or their progeny, cotton producers would still have the same options currently available to them for the control of lepidopteran insect pests of cotton. Several chemical insecticides and biopesticides are available for the control of lepidopteran insect pests of cotton. Appendix C lists some of the other plant-incorporated-protectants and chemical

insecticides that can be used for the control of these target pests along with their environmental fate and toxicity profiles. Some of the chemical insecticides are more toxic to nontarget organisms such as fish, birds, bees, and small mammals, and are more likely to have negative effects on humans. But cotton farmers might also choose biopesticides specific to lepidopterans, e.g. those based on formulations of B.t. in microbial preparations such as Lepinox (see http://www.epa.gov/pesticides/biopesticides/ingredients/index.htm for a list of biopesticides) or other B.t. cotton varieties. B.t. cotton varieties referred to as Bollgard® (sometimes referred to as Bollgard I) or NuCotn express the insecticidal protein Cry1Ac, and Bollgard II®, expresses both Cry1Ac and Cry2Ab. These cotton varieties developed by Monsanto were derived from transformation events previously deregulated by APHIS, and they also deter feeding of lepidopteran insects. The status of the EPA registrations and tolerance exemptions for these products is updated by the EPA at their website (see http://www.epa.gov/pesticides/biopesticides/pips/pip_list.htm.). Review of these plant-incorporated-protectant B.t. cotton varieties by APHIS and the EPA have shown them to have no plant pest effects or unreasonable adverse effects on the environment (see USDA, APHIS decision documents for petitions 00-342-01p and 94-308-01p available at http://www.aphis.usda.gov/brs/de_reg.htm., and US EPA, 2001 and 2002) . Use of B.t. cotton varieties has increased quickly in the United States, expanding from 15 percent of cotton acreage planted in 1997 to 37 percent in 2001 (Fernandez-Cornejo and McBride, 2002, see http://www.ers.usda.gov/publications/aer810/).

The no-action alternative would preclude the commercial development and registration of stacked Cry1F/Cry1Ac cotton varieties based on Cotton Event Cry1F and Cry1AC that might otherwise potentially extend the usefulness of the first generation B.t.-cotton varieties by slowing the development of resistance to the Cry1Ac protein and Cry2Ab proteins produced in these varieties in target insect pests. However, granting nonregulated status does not guarantee the extent to which a new plant line would be adopted by growers.

B. Alternative B: Approval of Either Petition or Both Petitions in Whole

APHIS may grant a petition for nonregulated status in whole or in part. By granting the subject petitions in whole, APHIS would grant the petitions as requested, i.e., that Cotton Event Cry1F and Cotton Event Cry1Ac and cotton progeny derived from either or both of these should no longer be considered regulated articles. The APHIS assessment of the environmental impacts of such determinations are discussed in the following sections. Environmental impacts of unrestricted cultivation of Cotton Events Cry1F and Cry1Ac are compared to impacts of current practices in the cultivation or distribution of cotton not regulated under 7CFR part 340.

1. Plant pathogenic properties

APHIS considered the potential for the transformation process, the introduced DNA sequences or their expression products to cause or aggravate disease symptoms in Cotton Events Cry1F and Cry1Ac or their progeny or in other plants. We also considered whether data indicate that unanticipated plant pest effects would arise from cultivation of Cotton Events Cry1F, Cry1Ac or their progeny. APHIS considered information from the scientific literature as well as primary observations made by the developer when the plants were grown in the environment.

Recipient organism

The starting plant material for the genetic transformation for each of the Cotton Events Cry1F and Cry1Ac was the cotton cultivar 'Germain's Acala GC510' (*Gossypium hirsutum* L.) released in the USA in 1984 by Germain's Agribusiness, Inc. This commercially acceptable Acala type cotton cultivar will be referred to as GC510.

Transformation system

The transformation system for Cotton Events Cry1F and Cry1Ac employed an Agrobacterium – mediated transformation method that utilized separate binary plasmid vectors, pGSV71 and pMYC3006, respectively, carrying the foreign genes of interest within a disarmed transfer DNA (T-DNA) from *Agrobacterium tumefaciens*. The disarmed T-DNA lacks the hormone genes from this pathogen that otherwise cause crown gall disease symptoms. *Agrobacterium*–mediated transformation is a well characterized technique that has been used for the transformation of plant cells for over two decades. Following incubation of GC510 cotyledon segments with the *Agrobacterium* DNA vector, plant tissue that took up the foreign T-DNA insert was selected on medium containing the herbicide glufosinate ammonium and the *Agrobacterium* was killed with the antibiotic carbenicillin. This transformation technique prohibits further transfer of DNA or the development of crown gall disease as a result of the *Agrobacterium*.

DNA sequences inserted into Cotton Event Cry1F and Cotton Event Cry1Ac

Cotton Event Cry1F: The Mycogen/Dow petition 03-036-01p (including their Sept. 15, 2003 response to the technical review letter dated July 14, 2003 from APHIS) provided data to support the conclusion (pg. 20 of the petition) that Cotton Event Cry1F contains a single integration that contains all of the transgene elements comprised within the T-DNA of the transforming plasmid pAGM281. This inserted DNA consists of the following genetic elements (described in detail in Section IV and Table 2, pg. 17 of the petition): 1) the mannopine synthase promoter including 4 copies of the octopine synthase enhancer, referred to as (4OCS)Δmas2', both of which are derived from the crown gall tumor inducing (Ti) plasmid from *A. tumefaciens*; 2) a synthetic plant-optimized gene encoding a full length chimeric version of Cry1F originally from *B.t.* var. *aizawai* (referred to as cry1F(synpro) of which the first 604 amino acids are comprised of the toxic portion of Cry1Fa2, and the remaining 544 amino acids are comprised of 36 amino acids from the carboxyterminal domain of Cry1Ca3, derived from *B.t.* var. *aizawai* PS81I, followed by 508 amino acids from the carboxyterminal domain of Cry1Ab1 derived from *B.t.* var. *berliner* 1715, which

together include the carboxyterminal portion that is removed following protease cleavage in the alkaline midgut of insects; 3) a bi-directional terminator containing the polyadenylation signal from open reading frame 25 (ORF25 polyA) from *A. tumefaciens*, 4) a synthetic plant-optimized version of the *pat* gene encoding the enzyme phosphinothricin acetyltransferase from *Streptomyces viridochromogenes* that confers resistance to the herbicide glufosinate-ammonium; 5) a promoter, first exon (untranslated enhancer) and first intron from the *Zea mays* ubiquitin 1 gene referred to as UbiZm1. Data presented also indicate there is a second copy of the ubiquitin 1 promoter element connected to a partial copy of the *pat* gene that is not contiguous with the ORF25 Poly A signal, and that expression from this gene was low at the RNA transcript level and undetectable at the protein level.

Cotton Event Cry1Ac: The Mycogen/Dow petition 03-036-02p (including their Sept. 15, 2003 response to the technical review letter dated July 14, 2003 from APHIS) provided data to support the conclusion (pg. 21 of the petition) that Cotton Event Cry1Ac contains a single integration that contains all of the transgene elements comprised within the T-DNA of the transforming plasmid pMYC3006. With the exception of the Cry1F gene, the genetic elements are the same as those included in Cotton Event Cry1F described in the previous paragraph, except that the promoter elements for the two genes are switched. This inserted DNA consists of the following genetic elements in the order indicated (described in detail in Section IV and Table 2, pg. 18 of the petition): 1) UbiZm1 promoter, first exon, and intron; 2) a synthetic plant-optimized gene encoding a full length chimeric version of Cry1Ac1 originally from *B.t.* var. *kurstaki* strain HD73 (referred to as *cry1Ac* (synpro) of which the first 612 amino acids are comprised of the toxic portion of Cry1Ac1, and the remaining 544 amino acids are comprised of the same carboxyterminal domain sequences from Cry1Ca3 and Cry1Ab1 as described above for the Cry1F(synpro); 3) the bi-directional terminator ORF25 polyA; 4) the synthetic plant-optimized *pat* gene from *S. viridochromogenes*; and 5) the (4OCS)Δmas2' promoter/enhancer element from *A. tumefaciens.*

Data presented by Mycogen/Dow also show that a bacterial gene encoding resistance to the antibiotic erythromycin, which was present outside the T-DNA in the transforming plasmids pAGM281 and pMYC3006, was not transferred to Cotton Event Cry1F or to Cotton Event Cry1Ac (see pp 36-37 in both petitions 03-036-01p and 03-036-02p).

Of all of the DNA sequences inserted in the construction of Cotton Events Cry1F and Cry1Ac, only the promoter and enhancer elements from mannopine and octopine synthase and the ORF polyA termination sequences were derived from an organism known to be a plant pest, i.e., *A. tumefaciens*. These noncoding sequences are well characterized, both in their native organism and as part of recombinant DNA constructs used in plant engineering to facilitate the expression of the introduced genes. There are no data to suggest that these sequences cause plant disease or pose a plant pest risk in transgenic plants. Multiple generations of plants derived from Cotton Events Cry1F and Cry1Ac have been observed closely, and the petitioners have confirmed the expectation that these noncoding DNA sequences do not cause disease in the plants (see sections below for discussion of additional evaluations of the attributes of Cotton Events Cry1F and Cry1Ac plants).

None of the other donor organisms used as sources or as the basis for the DNA sequences engineered into the cotton to make Cotton Events Cry1F and Cry1Ac are organisms with demonstrated plant pest characteristics. Both *S. viridochromogenes* and *B. thuringiensis* are bacteria commonly found in soils around the world. *B. thuringiensis* (Bt) also occurs naturally on plants, but is not a plant pathogen. Different varieties of this bacterium produce a crystal protein that is toxic to specific groups of insects. *B. thuringiensis* strains have been used for decades in agriculture as the basis for microbial pesticide formulations (bacteria are grown in laboratories to prepare suspensions that can be applied to plant surfaces to deter plant eating insects).

Evaluation of intended effects in Cotton Events Cry1F and Cry1Ac: inheritance and expression of cry1F*(synpro), *cry1Ac*(synpro) *and pat

As expected, in both transformation events the genes conferring insect resistance and the selectable marker herbicide tolerant gene(s) are linked and co-segregate as a single dominant locus. All of the transgenes inserted are expressed as expected, and the proteins have the expected biochemical characteristics and confer the intended traits.

Inheritance of the transgenes: Data was provided that demonstrates stable co-segregation of the *pat* gene(s) with the *cry1F* (synpro) gene and *cry1Ac (synpro)* gene within a segregating generation of Cotton Event Cry1F and Cotton Event Cry1Ac, respectively. Furthermore, expression of the Cry1F (synpro) protein and Cry1Ac (synpro) protein, as measured by an immunoassay, was correlated with occurrence of their corresponding gene (see pp, 34-35, Fig. 10 and 11 of each subject petition). Chi square analysis of inheritance data from at least 3 advanced generations demonstrate that the *cry1F* (synpro) gene and the *cry1Ac* (synpro) gene are both inherited in a predictable manner consistent with a single locus dominant trait according to Mendelian genetics when Cotton Event Cry1F and Cotton Event Cry1Ac plants are crossed with other cotton plants (see pp. 38-39, and Table 7 of the respective subject petitions). This inheritance pattern is consistent with analysis of the DNA insert in these transformation events.

Analysis of expression of the introduced genes: Expression data from multiple samples collected from Cotton Event Cry1F and Cotton Event Cry1Ac plants grown in 2001 in replicated plots in six field sites representing a variety of agronomic practices and environmental conditions showed that the insecticidal proteins and selectable marker PAT protein were detectable in a variety of plant tissues (see pp. 40-42 and Tables 8-9 of each subject petition).

The insecticidal proteins were detected in all of the tissue fractions sampled with the exception of nectar (i.e, in young leaves, terminal leaves, flowers, squares, early bolls, pollen, seed, and whole plants and roots at the seedling, pollination and defoliation stages of growth;). Nectar samples were only collected at two sites for Cotton Event Cry1F and were not collected for Cotton Event Cry1Ac. The maximum mean values across plant tissues were 22.8 ng/mg tissue dry weight for Cry1F (synpro) protein and 1.92 ng/mg tissue dry weight for Cry1Ac (synpro). The relative levels in different tissue fractions varied somewhat between the two cotton transformation events. For example in pollen the level of Cry1F (synpro), though detected, was below the limit of

quantification, while Cry1Ac (synpro) was present at the higher levels similar to those found in leaves, flowers, squares and whole plant fractions. This variation may be due to differences in either the gene promoters used and/or the stability of these two proteins in the plant, as Cry1Ac (synpro) appears to be more sensitive to protease digestion. Values for the stacked product were comparable to the single event for both proteins.

PAT protein levels in both cotton transformation events were much lower than for the insecticidal proteins. In Cotton Event Cry1F, PAT was detected in at least some of the samples collected for all tissue fractions except for roots collected during pollination and nectar, while for Cotton Event Cry1Ac, PAT was also not detected in pollen. The highest mean value of PAT for all fractions tested was 0.51 ng/mg tissue dry weight in Cotton Event Cry1F and 0.11 ng/mg tissue dry weight in Cotton Event Cry1Ac. Since expression of this protein was only essential during the plant transformation process as a selectable marker, its expression level in whole plants is not important for the intended use of these products. PAT protein accumulation is often found to be low in transgenic plants, even in plants specifically marketed as glufosinate tolerant**.**

Biochemical characterization of the expressed proteins: Data presented in both petitions indicated that the insecticidal proteins Cry1F (synpro) and Cry1Ac (synpro) had the expected biochemical characteristics (see pp 43-50 in petition 03-036-01p for Cry1F (synpro) and pp. 43-51 in petition 03-036-02p for Cry1Ac (synpro)). The amino acid sequence deduced from the Cotton Event Cry1F plant DNA was used to confirm that the introduced sequence was identical to the expected sequence as determined from the transformation construct (see 03-036-01p pp 44-45 and pp 11-13 of the Mycogen/Dow letter to APHIS dated Sept. 15, 2003). Biochemical characterization of the expressed insecticidal proteins demonstrated that in leaf extracts the full-length protein is cleaved at the N-terminus removing the first 27 amino acids of the Cry1F (synpro) protein and the first 28 amino acids of the Cry1Ac (synpro) protein in Cotton Events Cry1F and Cry1Ac, respectively, and both proteins are also cleaved at the C-terminus leaving in both cases a non-glycosylated core protein with a molecular weight of 65 kDa which has been shown to possess insecticidal activity (see pp. 46-50). The predominant form of the Cry1F protein is essentially identical to that expressed from the truncated, 65 kDa protease-resistant core Cry1F protein (which ends at amino acid 605) that is expressed in the corn line 1507 which APHIS has already deregulated (petition 00-136-01p). Lower molecular weight forms of both proteins, particularly Cry1Ac (synpro), were also detected. These were most likely the result of further proteolysis, either in the plant or during extraction.

Biochemical analysis (western blots) of the PAT protein extracted from flower buds taken from Cotton Events Cry1F and Cry1Ac and the stacked cotton product demonstrated that it was of the expected molecular weight (see pp 51-52 in petition 03-036-01p and pp 52-53 in petition 03-036-02p). Although DNA analysis of Cotton Event Cry1F indicated the presence of both a complete and a partial copy of the *pat* gene, only one apparently full length version of the PAT protein was detected.

Analysis of the intended traits: Expression of Cry1F (synpro) and Cry1Ac (synpro) is expected to confer resistance to certain lepidopteran pests of cotton. Expression of the *pat* gene is expected to confer tolerance to the herbicide glufosinate ammonium as a selectable marker to facilitate selection of transgenic plants, as was demonstrated. Cotton Events Cry1F and Cry1Ac have been field tested in a variety of locations in 13 states that represent major cotton growing regions (see Appendix B of this EA for lists of field tests). Cotton Event Cry1F was also tested in Puerto Rico. The stacked product and nontransgenic parental cotton lines were also included in most of these tests. The number of years of field testing span from 1999 - 2002 for Cotton Event Cry1Ac, 2000-2002 for Cotton Event Cry1F, and 2001-2002 for the stacked product. Field data reports submitted from these field tests include summaries of observations regarding the pest susceptibilities of the transgenic cotton lines compared to the non-transgenic parental line and indicate no differences in pest susceptibilities except to targeted lepidopteran pests and some level of tolerance to the herbicide glufosinate ammonium. In addition, during 2000-2001 studies were conducted to compare the transformed Cotton Events to the non-transgenic recurrent parent PSC355 to which both transformation events were crossed, with respect to their efficacy in resistance to certain lepidopteran pests. The results as stated in the petitions are summarized in the table below.

Lepidopteran pest	Cotton Event Cry1F	Cotton Event Cry1Ac
tobacco budworm (*Heliothis virescens*)	Good efficacy	Good efficacy
beet armyworm (*Spodoptera exigua*)	Good efficacy	Moderate efficacy
soybean looper (*Psuedoplusia includens*)	Good efficacy	Moderate efficacy
cotton bollworm (*Helicoverpa zea*)	Moderate efficacy	Good efficacy
pink bollworm (*Pectinophora gossypiella*)		Good efficacy

The field data reports also report various levels of resistance of these Cotton Events to other lepidopteran pests of cotton, including fall armyworm and cabbage looper, and a report submitted on efficacy of the stacked product indicates that, as expected, it provides some control over all these lepidopteran pests (Pellow, 2002 in Appendix 2 of both petitions).

Evaluation of possible unintended plant pest effects in Cotton Events Cry1F and Cry1Ac
Expression of the foreign insecticidal proteins and PAT are not expected to cause plant disease or influence susceptibility of Cotton Events Cry1F and Cry1Ac to plant pathogens or non-lepidopteran pests. Observational data in field data reports for the field tests of these Cotton Events (listed in Appendix B of this EA) confirmed that these plants were no more susceptible to pathogens and pests of cotton observed during these field trials, other than the expected resistance to certain lepidopteran pests, as compared to non-transgenic parental cotton lines. Pathogens or disease agents observed include seedling diseases (seed rot, root rot, or damping-off including Rhizoctonia), Fusarium and Verticillium wilts, boll rot, Phomopsis leaf spot and

Bacterial Blight. Non-target pest or beneficial arthropods observed at various locations include for example cotton and cabbage aphids, thrips, cotton fleahopper, whiteflies, spider mites, ants, striped beetles, unspecified bollweevils, Tarnished Plant Bug (*Lygus* spp.), stinkbugs, and predatory insects including Lady beetles, Green lacewing, Assassin Bug, Big-eyed Bugs (*Geocoris* spp.), Minute Pirate Bug (*Orius* spp.), and Damsel Bugs (*Nabis* spp.).

In order to evaluate possible unintended effects of the transformation process, including tissue culture, APHIS considers a wide range of plant attributes in much the same way that traditional plant breeders evaluate the offspring from traditional plant crosses or mutagenesis procedures. In addition to observations on pest and disease susceptibility, the petitions included data from agronomic trials conducted across the major regions of the US cotton belt that compare the subject Cotton Event Cry1F or Cry1Ac and the stacked product to the recurrent non-transgenic parent PSC355 with regard to various agronomic attributes or characteristics including aspects of growth habit, germination and emergence, vegetative vigor, flowering period, reproductive potential, and fiber quality. Depending on the characteristic examined, data was gathered from 15 to 20 locations, and means were calculated over locations from samples collected from 4 replicate plots per location (see pp 53-54 and Table 13 in petition 03-036-01p and pp. 54-55 and Table 14 in petition 03-036-02p, additional information (including statistical analysis of the means, and maximum and minimum values and standard deviations) was also supplied in the Mycogen/Dow letter of Sept. 15, 2003 to APHIS. Statistically significant differences were noted between the means across locations for PSC355 and Cotton Events Cry1F and Cry1Ac for the following attributes: number of vegetative bolts and vegetative branches per plant, field emergence, days to first flower, and in the case of Cotton Event Cry1Ac, percent germination in the cool vigor test and plant height. However, these differences were slight and in some cases were not significantly different in the stacked product. Low field emergence rates of Cotton Events Cry1F and Cry1Ac were attributed to the seed having been produced in a winter nursery resulting in seed of uneven quality, but percent total germination of seed harvested from the agronomic trials was not significantly different. Both Cotton Events Cry1F and Cry1Ac were also found to be significantly different from PSC355 in certain aspects of reproductive potential and fiber quality, but these differences are generally favorable and do not indicate a plant pest risk or diminish agronomic performance or value. Mean values for pounds of lint per acre were higher, though not significantly, for both Cotton Events Cry1F and Cry1Ac and the stacked product, and differences or improvements in fiber quality were attributed to the Acala cotton variety background in the transgenic lines. The field observations indicate that Cotton Events Cry1F and Cry1Ac are typical of traditional cotton in terms of growth and agronomic performance.

Mycogen/Dow also presented data collected from 6 locations in the major US cotton-producing regions on the composition of the seeds or processed fractions derived therefrom (i.e. kernels, hulls, toasted meal, and refined oil) (see petition 03-036-01p pp.55-60 and Tables 14-19, and petition 03-036-02p pp 56-61 and Tables 15-20, and Phillips et al. 2002 in Appendix 2 of both petitions). Data demonstrate that the percent ash, total fat, moisture, protein, carbohydrates, calories, and fiber content in the various fractions in which they were analyzed were not significantly different between Cotton Events Cry1F and Cry1Ac and their non-transgenic recurrent parent counterparts. Naturally occurring toxicants and antinutrients (gossypol, tocopherols, phytic acid and the clyclopropenoid

fatty acids) were also analyzed in some of these same fractions as well as in terminal leaves and squares of the transgenic and control plants and were found to be similar and/or within the acceptable ranges reported in the literature for these components.

In evaluating the range of plant attributes, Cotton Events Cry1F and Cry1Ac and the stacked product appear to be similar to the nontransformed recurrent parent counterpart cotton line except for their intended enhanced resistance to feeding damage from some lepidopteran pests of cotton and some tolerance to the herbicide glufosinate. No intended or unintended plant pest effects have been observed in these Cotton Events, and APHIS can not envision any plant pest effects arising from a determination that Cotton Events Cry1F and Cry1Ac should no longer be considered regulated articles under the APHIS regulations found at 7CFR Part 340.

2. Potential Impacts based on the relative weediness of Cotton Events Cry1F and Cry1Ac compared to currently cultivated cotton varieties.

APHIS evaluated whether Cotton Events Cry1F and Cry1Ac would be any more likely to become a weed than their nontrangenic counterpart, or than other cotton varieties currently offered for commercial use. The cultivated cotton from which these Cotton Events are derived, *Gossypium hirsutum*, is not typically considered a weed species in the United States or other countries (Reed, 1977; Muenscher, 1980; Holm et al., 1977, 1997, USDA, NRCS, 2001) nor is it listed in the Weed Science Society=s Composite List of Weeds (1989). However, cotton has some characteristics as a weed, and the Southern Weed Science Society lists *G. hirsutum* as a potential weed in southern Florida (Southern Weed Science Society, 1998). Without human intervention, such as the typical agricultural practices, the cotton plant is a perennial, surviving many years if conditions allow. Cotton does not tolerate cold conditions, and only Hawaii, southern Florida, and Puerto Rico remain warm enough to allow cotton plants to survive the winter (Smith and Cothren, 1999).

APHIS believes that data presented in the Mycogen/Dow petitions on the agronomic properties and pest susceptibility of Cotton Events Cry1F and Cry1Ac substantiate that these transgenic plants are similar in growth and development to the parental cotton line. As noted some statistically significant differences were noted between the means across field test locations for PSC355 and Cotton Events Cry1F and Cry1Ac. However, these differences were slight and in some cases were not significantly different in the stacked product, and they would not be expected to increase weediness. Although numbers of vegetative branches and vegetative bolts were slightly higher, plant height was not increased, so there would not be a significant impact on the plants ability to competitively shade other plants. These Cotton Events did not exhibit a change in attributes that are characteristics of those of some of the worst or ideal weeds as described by Baker (1965 and 1974). There was no increase in percent germination under ideal conditions or under varied environmental conditions (cool and warm) and no significant increase in seed dormancy, nor was there an increase in resistance to seedling diseases, therefore seedling establishment is not enhanced. The number of days to flowering was slightly increased, not decreased. The reproductive potential of the Cotton Events and stacked product is similar to that of the nontransgenic recurrent parent, as the calculated

number of seeds per boll and numbers of bolls were not substantially different (see Mycogen/Dow letter of Sept. 15, 2003 to APHIS). There were also no substantial differences in antinutrients or toxicants that might affect interactions of these Cotton Events with other organisms that could aid in the dispersal of seed.

In addition to the results summarized above, APHIS notes that there have been no reports of increased weediness associated with other lepidopteran insect resistant or glufosinate tolerant cotton lines that have been deregulated**.** A comparison of environmental impacts of biotechnology derived and traditional cotton crops has not identified weediness associated with insect resistant cotton lines being grown in the U.S. (Carpenter *et al.*, 2002). Cotton Events Cry1F and Cry1Ac appear to pose no greater plant pest risk of weediness than that posed by traditional cotton cultivars.

3. Potential impacts from gene introgression from Cotton Event Cry1F and Cry1Ac to sexually compatible relatives.

Cotton Events Cry1F and Cry1Ac, like other cotton, can pass its traits to offspring by transmitting pollen to other plants which are sexually compatible, in this case, some species of the genus *Gossypium* (see Appendix A for a brief technical discussion of the biology and reproductive capability of cotton). Recently, EPA has provided an even more detailed overview of the genus *Gossypium* in Biopesticides Registration Action Document (http://www.epa.gov/pesticides/biopesticides/reds/brad_bt_pip2.htm, see especially pages IIC7-IIC13 in US EPA, 2001).

APHIS considered whether such crosses are likely to occur when Cotton Events Cry1F and Cry1Ac are grown, and whether the offspring from such crosses are more likely to pose any greater risk of weediness than crosses of other cotton cultivars with these sexually compatible species.

The genus *Gossypium* contains approximately 50 species, of which generally four species are cultivated for the cotton fibers that are attached to the seeds. Cotton Event Cry1F is *Gossypium hirsutum*, the cotton species referred to as upland cotton. Most of the cotton grown in the United States is *G. hirsutum*, but Pima cotton (*G. barbadense* L.) is also grown. In addition to these cultivated species, there are two wild *Gossypium* species in the United States, *G. thurberi* and *G. tomentosum*, which are found in the mountains of southern Arizona and in Hawaii, respectively. Neither *G. thurberi* nor *G. tomentosum* are listed as weeds, either on the Federal or State lists of noxious weeds (see http://plants.usda.gov/cgi_bin/noxious.cgi?earl=noxious.cgi). An older literature citation lists *G. tomentosum* as a weed of unknown importance in its range (Holm et al.,1979).

Genetic incompatibility precludes successful crosses of *G. hirsutum* with *G. thurberi*, but the compatibility of crosses between *G. hirsutum* and *G. tomentosum* is less understood. Some researchers have speculated that crosses may have occurred in the evolution of *G. tomentosum*, but genetic exchange appears to be rare. Part of the rarity may be due to the fact that *G. hirsutum* is

largely self-pollinating rather than cross-pollinating. In addition, the pollinators of *G. hirsutum* tend to be bumblebees, whereas moths pollinate *G. tomentosum*. Also, *G. hirsutum* flowers are sexually receptive for pollination during the day, *G. tomentosum* compatibility is at night. APHIS has consulted with Dr. Derral Herbst, a prominent botanist in Hawaii with decades of experience and an author of the definitive "Manual of the Flowering Plants of Hawaii" recently revised in 1999 (personal communication with Bruce MacBryde, Jan. 30, 2004). Dr. Herbst, indicated that based on his field work over the years and on herbarium collections at the Bishop Museum, which houses the Hawaiian Biological Survey, he has not seen a hybrid between *G. tomentosum* and either of the cotton species which have naturalized there, *G. hirsutum* and *G. barbadense*. He was also of the understanding that genetic barriers between the species result in weak, sterile F2 generations.

Even in cases of complete genetic compatibility (*G. hirsutum* crossed with another *G. hirsutum*), successful outcrossing is severely limited when the plants are separated by more than 660 feet. In experiments designed to detect gene flow in Mississippi, detectable gene flow was very low (less than 1%) when *G. hirsutum* plants were 25 meters apart (Umbeck, 1991). Cotton breeders and seed producers routinely use field data to decide on the isolation distances for the production of certified and foundation cotton seeds (660 and 1320 feet, respectively).

In sum, APHIS believes that it is very unlikely that Cotton Events Cry1F and Cry1Ac will successfully cross with wild sexually compatible relatives when grown in the United States. In the unlikely event that such crosses do occur, however, the lack of increased weediness in these transgenic cotton events (described in the section above) suggests that any offspring would be unlikely to pose an increased risk of weediness.

Because it is unlikely that *G. hirsutum* will readily cross with *G. thurberi* and *G. tomentosum*, it is unlikely that the genes encoding the insecticidal proteins and PAT protein will introgress from Cotton Events Cry1F and Cry1Ac into *G. thurberi* and *G. tomentosum*. In the registration requirements for other Bt-cotton varieties (Bollgard and Bollgard II), the EPA stipulated geographic restrictions to mitigate gene flow to sexually compatible relatives in parts of the United States where *G. thurberi* and *G. tomentosum* are found, imposing conditions based on reproductive compatibility in crosses of *G. hirsutum* to other *G. hirsutum* (US EPA, 2001 and 2002). As summarized above, however, such crosses between the cultivated and wild cottons do not appear to occur in nature. There are no reports of intermediate cotton types that one would expect in the areas where *G. hirsutum* has been grown in proximity to *G. thurberi* and *G. tomentosum*.

Outcrossing considerations may be different in other parts of the world. For example, other species which might potentially intercross with *G. hirsutum* cultivars include *G. mustelinum* in northeastern Brazil, and *G. lanceolatum* in mid-Mexico (Fryxell 1979). Other Old World Gossypium cottons are diploid, as are the other five genera of cotton relatives among the Gossypieae Tribe (Fryxell, 1979). The likelihood of successful intercrossing with these diploid species may be quite low because of the production of triploids that are likely to be sterile. This is consistent with the fact that such intergeneric crosses have not been observed (Fryxell, 1979).

APHIS believes that gene flow from Cotton Events Cry1F and Cry1Ac to wild cotton relatives is not likely, and if it occurs, would not lead to increased weediness. On July 2001, EPA published its final FIFRA regulations regarding plant incorporated protectants, of which the Bt Cry proteins are an example (http://www.epa.gov/pesticides/biopesticides/pips/pip_rule.pdf). APHIS agrees with the EPA statement in its final rule on plant-incorporated protectants that Aweediness is generally thought to be due to a multiplicity of factors≅ (US EPA, 2001b). The National Research Council has also concluded that Agenetically modified crops are not known to have become weedy through the addition of traits such as herbicide and pest resistance≅ (National Research Council, 1989).

4. Potential impacts on nontarget organisms, including beneficial organisms and threatened and endangered species

APHIS evaluated the potential that Cotton Events Cry1F and Cry1Ac might have an adverse impact on populations of nontarget organisms, i.e., organisms other than the lepidopteran pests of cotton. As discussed in the previous section, APHIS considers it highly unlikely that the Bt insecticidal genes will be passed through natural crossing from Cotton Events Cry1F and Cry1Ac into wild relatives. Therefore, APHIS restricted its analysis of potential impacts to nontarget organisms, to those that could be exposed, directly or indirectly, to tissues of these cotton plants that express the insecticidal proteins or to active forms of these insecticidal proteins in the environment following release from these plant tissues. APHIS has prepared numerous environmental assessments on petitions for deregulations of plants, including cotton, expressing the PAT protein (see APHIS EA for petition 02-042-01p and other phosphinothricin–tolerant crops available at http://www.aphis.usda.gov/brs/de_reg.htm). Plants expressing this protein have been commercialized, and this protein has not been found to have adverse effects on wildlife or humans.

In addition to APHIS review of nontarget effects for petitions for nonregulated status, the EPA also evaluates potential nontarget effects in the course of its review for pesticide registrations. These data were included in Appendix 2 of the subject petitions to APHIS. Based on the data presented, and information in the scientific literature, EPA can mandate mitigation measures as part of the conditions for pesticide registration. To evaluate the potential non-target effects of the insecticidal proteins expressed in Cotton Events Cry1F and Cry1Ac, a standard battery of nontarget organisms were evaluated for their sensitivity by forcing them to ingest controlled amounts of test substance containing one or both of the insecticidal proteins Cry1F (synpro) or Cry1Ac (synpro) as expressed in cotton or from similar full-length chimeric constructs of Cry1F and Cry1Ac produced in a microbial source. Nontarget test organisms also included organisms that are found in or near to the agricultural environment in which cotton is grown.

To justify use of the different test substances, the petitions included data that establish that the insecticidal proteins Cry1F (synpro) and Cry1Ac (synpro) produced from the cotton plants had similar biochemical characteristics to the insecticidal proteins produced from the microbial source (*Psuedomonas fluorescens*) (see pp. 43-52 and Appendix 2 Vol. 8 Gao *et al.,* 2001 in 03-036-01p and pp. 43-51 Appendix 2, Vol. 6 and 7, Gao *et al.,* 2001 and 2002, respectively in 03-036-02p) .

There were some minor differences in amino acid sequence for Cry1F, primarily in the portion of the insecticidal protein that is proteolytically cleaved. There were also differences in the predominant molecular weight forms of the two insecticidal proteins from the different extracts, most likely due to proteolysis. Data presented in the petitions also demonstrate the bioactive equivalency of the insecticidal proteins derived from the two sources; they have similar potencies (as measured by growth inhibition and/or mortality) against insect species with different degrees of susceptibility (see pg. 61, Table 21 in 03-036-01p and pg. 62, Table 21 in 03-036-02p).

Selectivity and impacts of the insecticidal proteins to Lepitdoptera, including monarch butterflies
The Cry1 class of insecticidal proteins show the greatest activity against the Lepidoptera order of insects. This insecticidal activity is dependent upon binding to specific receptors present in the mid-gut of susceptible insects (Lambert, et al., 1996; Van Rie et al., 1990; Van Rie et al., 1989; Hoffmann et al., 1988a and 1988b; and Wolfersberger et al., 1986).

Data on susceptibility of insect species of different orders associated with cotton demonstrated the relative selectivity of the full-length chimeric versions of Cry1F and Cry1Ac insecticidal proteins (as expressed from the microbial source) to Lepidotera as compared to insects from other orders (see pg. 62 and Herman and Young, 1999 Appendix 2, Vol. 11 in 03-036-01p, and pg. 63 and Herman, 2001, Appendix 2, Vol.10 in 03-036-02p. The western tarnished plant bug (*Lygus hesperus*) and the boll weevil (*Anthonomus grandis grandis*), insects from the orders Heteroptera and Coleoptera, respectively, were 10 to 10,000 fold less susceptible to Cry1F than six of the seven lepidopterans tested, and cotton aphid (*Aphis gossypii*), from the order Homoptera, and boll weevil had significantly lower susceptibility to Cry1Ac than five of the six Lepidoptera tested.

APHIS evaluated the potential for nontarget lepidopterans (caterpillars) that feed on other plants besides Cotton Events Cry1F and Cry1Ac to be impacted by exposure to pollen drifting onto their food source. A 1999 study by Losey et al. reported results from a laboratory study in which monarch butterfly caterpillars died after eating corn pollen that expressed a Bt Cry1Ac protein. Considerable controversy followed about the potential effects that might arise when nontarget insects ingest plant pollen in which the Bt insecticidal protein is expressed. A series of subsequent studies concluded that monarch butterflies are unlikely to be significantly affected under conditions found in the agricultural and nonagricultural environments which they inhabit. This conclusion is consistent with the findings of several scientists, which were published as several reports in the Proceedings of the National Academy of Sciences (PNAS), and summarized in an accompanying risk assessment by Sears et al. (2001).

In the case of Cotton Event Cry1F and Cry1Ac, the petitions presented data and information that indicate that effects to monarch butterfly are not expected to be significant. Whereas corn plants are wind pollinated and disperse pollen on surrounding vegetation that might be ingested by nontarget organisms, cotton plants are primarily bee-pollinated which largely restricts pollen to the cotton flower. Monarch butterflies are also not likely to be exposed to the insecticidal proteins expressed by these cotton plants, because the primary geographic range and habitats for monarch butterflies and cotton cultivation do not coincide. Bridging from studies conducted on growth reduction

through dietary exposure of monarch butterfly larvae individually to Cry1F and Cry1Ac, conducted by Hellmich et al., 2001, the dietary concentration through exposure to pollen on leaf surfaces necessary for a 50% growth reduction in monarch butterfly larvae is greater than 450,000 times the estimated environmental concentration (EEC) of pollen from Cotton Event Cry1F and greater than 10 times the EEC of pollen from Cotton Event Cry1Ac (see pg. 64 in 03-036-01p and pp. 65-66 in 03-036-02p).

Potential Impact on Other Non-target Species

The Cry1F (synpro) and Cry1Ac (synpro) insecticidal proteins are not expected to adversely affect most other invertebrates and all vertebrate organisms, including non-target birds, mammals and humans. The toxicity and specificity of the lepidopteran specific Cry proteins is associated with their solubilization and proteolytic activation in the insect midgut, and their binding to specific cell membrane receptors present on the brush border membrane vesicles present in the midgut of susceptible insects (Van Rie, et al. 1989 and 1990). To evaluate the potential of Cotton Events Cry1F and Cry1Ac to have damaging or toxic effects on representative terrestrial and an aquatic species, APHIS evaluated data from a series of ecological toxicology experiments. The test organisms were mice, bobwhite quail, the fresh water species rainbow trout and *Daphnia magna,* respectively, and several invertebrate beneficial organisms including: soil-dwelling collembola (springtails) and earthworms, larval honeybees, predacious ladybird beetles and green lacewing larvae, and parasitic wasps.

For most of the nontarget organisms, data were presented in the petitions in which these non-target organisms were exposed to high doses of microbially-derived full-length chimeric proteins of Cry1F and Cry1Ac, alone and/or in combination with each other. Bobwhite quail and rainbow trout acute dietary toxicity studies were conducted with a diet consisting of 10% cottonseed meal prepared only from the stacked product. The lowest observed effect levels for Cry1F and Cry1Ac from these studies were compared to the high end exposure estimates (HEEE) for the Cry1F (synpro) and Cry1Ac (synpro) toxins in various plant tissues or their estimated environmental concentrations (EEC) in soil in Table 21 and 22, respectively in the Mycogen/Dow letter to APHIS submitted Sept. 15, 2003. Based on the results of these studies, no adverse effects on growth or increased mortality is expected at the maximum concentrations of these toxins to which the various test organisms could normally be exposed in the environment. For most species, there was at least a 5 fold margin of safety between the lowest observed effect level and the HEEE or EEC for the Bt toxins. The only exceptions were of dietary toxicity of Cry1F to honeybees and Cry1Ac to green lacewing (for which the LC_{50} was only 3 fold and 2 fold higher, respectively, than the HEEE).

The appropriateness of the methodology for the green lacewing acute toxicity study is questionable. The August 2002 FIFRA Scientific Advisory Panel (SAP) for B.t. corn noted concerns regarding this type of green lacewing study (Lewis, 2002). The SAP questioned the availability of Cry proteins to green lacewings when presented in a moth egg diet because the protein probably absorbs to the egg so only a small fraction of protein is contacted by larvae. The SAP recommended testing an alternate natural enemy such as the minute pirate bug (*Orius insidiosus*). Hemiptera such as the

minute pirate bug are also relatively important natural enemies or predators in cotton fields. Therefore, the EPA will be requiring an additional Tier 1 nontarget insect test with the minute pirate bug be conducted with the Cry1F and Cry1Ac proteins as a condition of full pesticide registration of these proteins as expressed in the Mycogen/Dow stacked cotton product (Leonard Cole, EPA, BPPD, personal communication to Susan Koehler, USDA, APHIS, Jan. 28, 2004). APHIS notes that previous studies with truncated Cry1F and Cry1Ac in support of previous corn and cotton petitions for deregulation, respectively, have not shown significant toxicity to honey bees or lacewing larvae.

Green lacewing, like other predatory insects is not expected to be directly exposed to the insecticidal proteins expressed in the subject Cotton Events. Little impact is expected for these species other than a possible shift to non-lepidopteran prey since lepidopteran populations in these Cotton Events are expected to be reduced. A two year, two location field study submitted by Mycogen/Dow did not detect any consistent major negative effects on non-target arthropod abundance (including green lacewings) in the stacked cotton treated with recommended insecticides to control non-lepidopteran pests as compared to non-transgenic cotton similarly treated or treated with conventional insecticides to control all insect pests including lepidopterans (see Mycogen/Dow letter to APHIS dated November 25, 2003 and the included study by Storer, 2003).

Appendix C of this environmental assessment is a summary table in which Cotton Events Cry1F and Cry1Ac are compared to other Bt cotton varieties that are commercially available in the United States (Bollgard and Bollgard II) and conventional chemical insecticides used to control lepidopteran pests of cotton. The comparison encompasses environmental fate and potential nontarget effects. In general, the toxicity data supporting these Cotton Events compare favorably to these products with respect to the potential for harm in the environment. For example, the pyrethroid insecticides are very highly toxic to aquatic organisms, many of the recommended pyrethroid and organophosphate insecticides and Spinosad are highly toxic to bees, and some of the organophosphates are highly toxic to mammals.

Potential impact on threatened and endangered species.

APHIS also considered the potential impact that nonregulated status of Cotton Events Cry1F and Cry1Ac might have on species which are on the Federal List of Threatened and Endangered Species. The incorporation of another type of Bt-cotton into cotton production may further the reduction of chemical pesticide use and the concomitant potential for negative impact to nontarget species via spray drift, bioaccumulation in food chains, and the contamination of surface and groundwater sources. APHIS did not focus its analysis extensively on such potential benefits, but examined the potential harm that might result from threatened and endangered species which are similar to the target insect pests and therefore likely to be sensitive to Cry1F (synpro) and Cry1Ac (synpro) when ingested. The threatened and endangered species most likely to be negatively affected by these proteins would be lepidopteran insects. Since it is not possible to use such species to quantify sensitivity to these Bt toxins, the APHIS evaluation started with the assumption of some toxicity and focused instead on whether it is likely that these species would be exposed to the toxins expressed in the subject transgenic cotton lines. Exposure of these species is only likely if the

species occur in the areas where cotton is grown, because cotton plant parts (seeds, pollen, crop debris) are not readily transported long distances without the intervention of humans.

The APHIS environmental assessment for the petition (00-342-01p) for deregulation of another Bt cotton, Bollgard II, which expresses both Cry1Ac and Cry2Ab examined the potential impacts on threatened and endangered species as did EPA's Biopesticides Registration Action Document for this product (see http://www.epa.gov/pesticides/biopesticides/ingredients/tech_docs/brad_006487.pdf). No new listed species have been identified that would be expected to be impacted. In the states which grow cotton, only California, Florida, and North Carolina have lepidopteran species that are on the Federal endangered species list. EPA and APHIS agree that these species do not feed on cotton, and their habitats do not overlap with cotton fields, furthermore, cotton pollen is heavy and is not expected to drift into these habitats in sufficient quantities onto host plants of the larvae forms of these species to have an effect.

Of the 15 California species, 13 are found in habitats which are far from the cotton growing areas in the Central Valley of California. Only one species, the Quino Checkerspot (*Euphydryas editha quino*) has populations in a cotton producing county. This Nymphalid butterfly is found in both upland sage scrub or chaparral communities and in meadows (Fish and Wildlife Service, 2001). Its host plants, the dotseed plaintain and the exerted Indian paintbrush both are adequate hosts for the larvae only in late winter and spring, but in the summer the vegetation mostly dies back. The adults emerge in early or midspring, and lay eggs which continue to grow until the summer dries the vegetation. A larval diapause occurs until the late winter and the host plants again flourish, until pupation occurs. It is likely that the insects would not commonly overlap with cotton cultivation, although in some years this might occur. Meadows in the vicinity of cotton and other agricultural production are likely to have been used for growing crops, and that is one reason why this insect has become endangered. Thus, geographic isolation is likely to prevent Cotton Events Cry1F and Cry1Ac from impacting this butterfly. The Fish and Wildlife Service has not described any agricultural impact on the populations of the Quino Checkerspot butterfly except the impact of livestock which trample the insect=s host plants (Fish & Wildlife Service, 1997).

A second endangered lepidopteran species in California, the Kern Primrose Sphinx (*Euproserpinus euterpe*), may occupy habitat near cotton cultivation sites in Kern County, but this moth has not been detected since 1982. It was formerly collected within southern Kern County on a single ranch (see EPA assessment of threatened and endangered species for Bollgard II cotton http://www.epa.gov/pesticides/biopesticides/ingredients/tech_docs/brad_006487.pdf) . It=s host plant is evening primrose, *Camissonia* spp., which are distributed throughout Southern California and beyond. APHIS does not believe that Cotton Events Cry1F or Cry1Ac would have an impact on the Kern Primrose Sphinx.

In North Carolina, another endangered butterfly, the St. Francis Satyr (*Neonympha mitchellii francisci*) is known, although cotton cultivation near its known habitat is unlikely. This butterfly

lives in the boggy areas and wide wet meadows of the Ft. Bragg military base (Fish & Wildlife Service, 1994), an area where cotton cultivation is unlikely.

In Florida, the Schaus swallowtail (*Heraclides aristodemus ponceanus*) is a subtropical species which lives in the far southern portion of the state. It is most commonly found on Elliot Key and North Key Largo. Cotton is not cultivated in this region, so exposure is very unlikely.

APHIS also considered threatened and endangered species other than lepidopterans. The petitions provided data which support the conclusion that the Bt proteins expressed in the subject Cotton Events are not toxic to invertebrates other than lepidopterans. Data also corroborated that they are relatively nontoxic to vertebrates (e.g. fish, birds, and mammals). These analyses are part of the EPA=s registration review that would be required before the stacked cotton product, currently under consideration for pesticide registration, could be sold as an insect resistant cotton variety. The EPA concurs with APHIS assessment that the insecticidal proteins expressed in the subject cotton events will pose no threat to threatened and endangered species (Leonard Cole, EPA, BPPD, personal communication to Susan Koehler, APHIS on Jan. 28, 2004).

In total, these analyses, and the data submitted by Mycogen/Dow and information in the scientific literature suggest that Cotton Events Cry1F and Cry1Ac and their stacked product should not pose a significant risk of harm to nontarget organisms.

5. Potential Impacts on Biodiversity

After careful evaluation, APHIS believes that Cotton Events Cry1F and Cry1Ac exhibit no traits that would cause increased weediness, that cultivation of these Cotton Events or the stacked product should not lead to increased weediness of other cultivated cotton or other sexually compatible relatives, and is unlikely to harm non-target organisms common to the agricultural ecosystem or threatened or endangered species recognized by the U.S. Fish and Wildlife Service. Based on this analysis, APHIS believes that it appears unlikely that deregulation of Cotton Events Cry1F and/or Cry1Ac would pose a significant impact on biodiversity.

6. Potential Impacts on Agricultural and Cultivation Practices

APHIS considered the potential impacts, including potential cumulative effects, of Cotton Events Cry1F and Cry1Ac and the stacked product on current agricultural practices in the United States. The potential impacts on organic farming and on minorities and children were also considered

Impacts on current agricultural practices

The comparative environmental impacts and impacts on agricultural practices from biotechnology-derived and traditional crops, including cotton were summarized in a report by Carpenter et al., 2001, published by the Council for Agricultural Science and Technology. In 1996, genetically engineered varieties called Bollgard were commercialized which utilize the Cry1Ac protein to deter

feeding damage from lepidopteran insect pests. The Economic Research Service of the USDA reports that in the year 2000 an estimated 35% of cotton acreage in the United States was planted with this approved variety (http://www.ers.usda.gov/Briefing/AgChemicals/Questions/bioqa1.htm). This variety provides excellent control of tobacco budworm and pink bollworm, good control of cotton bollworm (CBW), and fair to poor control of other pests. In some cases Bt cotton varieties based on the Cry1Ac protein alone must be supplemented with chemical insecticides to provide adequate control of bollworm and other pests (Godfrey *et al.,* 2000, Hardee, *et al.,* 2001), but numerous studies have demonstrated an overall reduction in insecticide sprays for lepidopteran pests as a result of the introduction of these Bt cotton varieties in the U.S. (US EPA, 2001, Bt cotton reassessment document, Table E.13, Gianessi and Carpenter, 1999).

Bollgard II cotton was developed by retransforming Bollgard to contain an additional insecticidal protein, Cry2Ab. This stacked product improves control of the CBW and expands the spectrum of insect control to additionally include the fall armyworm, beet armyworm, cabbage loopers, and the soybean looper. Because it contains two genes for lepidopteran resistance, instead of one, it is expected to slow the potential development of resistance in lepidopterans to the insecticidal proteins. Bollgard II became commercially available only last growing season (2003). It is expected to largely replace Bollgard as the preferred lepidopteran resistant cotton variety. As described above, the stacked product, which Mycogen/Dow intends to market as Widestrike™ developed from the Cotton Events Cry1F and Cry1Ac, is reported to provide control over a range of lepidopteran insects similar to Bollgard II. It is anticipated if deregulation is granted for Cotton Events Cry1F and Cry1Ac and Widestrike™ receives full pesticide registration from the EPA, that adoption rates for Widestrike will be similar to Bollgard II, and eventually both might replace Bollgard. The commercial use of such varieties may enable a continued reduction in the use of insecticides to control lepidopteran pests of cotton and improve options for the delay of resistant lepidopterans and their management.

The pesticidal use of Cotton Event Cry1F and traditional chemical pesticides in cotton cultivation are regulated by the EPA. During the pesticide registration process the EPA reviews the use of Insect Resistance Management (IRM) strategies to extend the useful life of transgenic plants with plant-incorporated protectants used in plants such as Bt-cotton. So far no populations of lepidopteran pests of cotton have developed resistance to the Bt toxins as a result of the deployment of Bt cotton or Bt corn varieties in the United States. In fact, use of Bt cotton in Arizona has been associated with regional declines in one of the target pests, pink bollworm, which may faciliate the use of even larger refuges for IRM (Carrière, *et al.*, 2003, Shelton *et al.*, 2002). The EPA is convening a scientific advisory panel in June 2004 to address IRM strategies for Bt cotton, including those previously registered as plant incorporated protects by the EPA and those that are pending registration, including the pending Cry1F (synpro)/Cry1Ac (synpro) plant-incorporated protectant as expressed in the Mycogen/Dow Widestrike cotton (Leonard Cole, EPA, BPPD, personal communication to Susan Koehler, Jan. 28, 2004). IRM requirements for currently registered Bt cotton are described by the EPA in their Bt crop reassessment document "Biopesticides Registration Action Document – Bacillus thuringiensis Plant-Incorporated Protectants" dated October 16, 2001, available at

http://www.epa.gov/pesticides/biopesticides/pips/bt_brad2/6-cotton.pdf. (US EPA, 2001). Mycogen/Dow has proposed to adopt the same IRM strategy for Widestrike as is currently in place for the other Bt cottons (Leonard Cole, EPA, BPPD and Irene Gatti, Dow, personal communication to Susan Koehler, Jan. 28, 2004). It is expected that EPA and Economic Research Service of the USDA will continue to monitor the use of such products to determine impacts on agricultural practices.

The glufosinate herbicide tolerance trait used as a selectable marker in the subject Cotton Events should not have a significant adverse impact on agricultural practices. Mycogen/Dow has stated that it is not their intent to market the stacked Cry1F (synpro)/Cry1Ac (synpro) cotton product as tolerant to glufosinate, and the EPA has confirmed this (Leonard Cole, EPA, BPPD, personal communication to Susan Koehler, APHIS, Jan. 28, 2004). The petitioners have indicated that if some farmers experience difficulty controlling volunteers of the stacked cotton variety in subsequent crops because they are using a glufosinate-ammonium based herbicide in glufosinate-tolerant (phosphinothricin tolerant) crops (e.g. corn or soybeans which have previously been deregulated by APHIS), they could address the issue through grower communications and/or product labeling.

Potential impacts on organic farming

It is not likely that organic farmers, or other farmers who choose not to plant transgenic varieties or sell transgenic grain, will be significantly impacted by the expected commercial use of products derived from Cotton Events Cry1F and Cry1Ac since: (a) nontransgenic cotton will likely still be sold and will be readily available to those who wish to plant it; (b) farmers purchasing seed will know this product is transgenic because it will be marketed and labeled as *Bt Cry1F/Cry1Ac* lepidopteran resistant, and, based on the IRM plan, farmers will be educated about recommended management practices (Leonard Cole, EPA, BPPD personal communication with Susan Koehler, Jan. 28, 2004).

Several transgenic cotton varieties resistant to lepidopteran insects are already in widespread use by farmers. Varieties derived from Cotton Events Cry1F and Cry1Ac should not present new and different issues than those with respect to impacts on organic farmers. APHIS has considered that it is possible that the genes from these Cotton Events could move to cotton in an adjacent field via cross-pollination. All cotton, whether genetically engineered or not, can transmit pollen to nearby fields, and a very small influx of pollen originating from a given cotton variety does not appreciably change the characteristics of cotton in adjacent fields. As described previously in this assessment, the rate of cross-pollination from one field to another is expected to be quite low, even if flowering times coincide. The frequency of such an occurrence decreases with increasing distance from the pollen source such that it is sufficiently low at 1320 feet away to be considered adequate for production of even the most restrictive standard for foundation cotton seeds (see footnote 19 for the table found at http://www.aphis.usda.gov/biotech/isolate.html). Organic cotton growers could use isolation distance or differences in planting time to minimize the potential for any unwanted outcrossing of transgenic cotton to their crop.

Potential impacts on humans, including minorities, low income populations, and children

In accordance with the directive specified in Executive Order 13045, APHIS has attempted to identify and assess environmental health or safety risks that might disproportionately affect children. APHIS also considered any possible adverse impacts on minorities and low-income populations as specified under Executive Order 12898 published February 11, 1994. Collectively, the available mammalian toxicity data and history of safe use of microbial *Bt* products and other cotton varieties expressing *Bt* proteins, supports the safety of Cotton Events Cry1F and Cry1Ac and their products to humans, including minorities, low income populations, and children who might be exposed to them through agricultural production and/or processing. No additional safety precautions would need to be taken in consideration of these groups. None of the impacts on agricultural practices described above are expected to have a disproportionate adverse effect on minorities, low-income populations, or children, and may in fact provide benefits. As noted above, if approved for cultivation, the stacked Widestrike cotton derived from Cotton Events Cry1F and Cry1Ac is expected to further decrease reliance on chemical insecticides used to control lepidopteran pests, some of which are less favorable with respect to environmental and human toxicity.

7. Potential impacts on raw or processed agricultural commodities.

Our analysis of data on agronomic performance, disease and insect susceptibility, and compositional profiles of the seeds and fiber indicate that Cotton Events Cry1F and Cry1Ac are similar to their non-transgenic parent counterpart and other cultivars of *G. hirsutum* grown in the United States. APHIS does not foresee either a direct or indirect plant pest effect on any raw or processed plant commodity.

8. Potential environmental impacts outside the United States.

APHIS has also considered potential environmental impacts outside the United States and its territories associated with a determination of nonregulated status for Cotton Events Cry1F and Cry1Ac. It should be noted that all the considerable, existing national and international regulatory authorities and phytosanitary regimes that currently apply to introductions of new cotton cultivars internationally, apply equally to those covered by an APHIS determination of nonregulated status under 7 CFR Part 340. Any international traffic in cotton subsequent to these determinations would be fully subject to national phytosanitary requirements and be in accordance with phytosanitary standards developed under the International Plant Protection Convention (IPPC). The IPPC has set a standard for the reciprocal acceptance of phytosanitary certification among the nations that have signed or acceded to the Convention (116 countries as of June, 2001). In addition, issues that may relate to commercialization and transboundary movement of particular agricultural commodities produced through biotechnology are being addressed in international forums and through national regulations. The Cartegena Protocol on Biosafety is a treaty under the Convention on Biological Diversity that established a framework for the safe transboundary movement, with respect to the environment and biodiversity, of living modified organisms (LMOs), which includes those modified through biotechnology. The protocol came into force on September 11, 2003 and 82 countries are parties to it as of Jan. 21, 2004 (see http://www.biodiv.org/biosafety/default.aspx.). Although the

United States is not a party to the CBD, and thus not a party to the Cartegena Protocol on Biosafety, US exporters will still need to comply with domestic regulation that importing countries that are parties to the Protocol have put in place to comply with their obligations. The first intentional transboundary movement of LMOs will require consent from the importing country under an advanced informed agreement (AIA) provision and the required documentation. To facilitate compliance with obligations to this protocol, the US Government is developing a website that provides the status of all regulatory reviews completed for different uses of the product. This data will be available to the Biosafety Clearinghouse database that contains regulatory decisions for LMOs that may be subject to the Biosafety Protocol.

APHIS continues to play a role in working toward harmonization of biosafety and biotechnology guidelines and regulations, including within the North American Plant Protection Organization (NAPPO), which includes Mexico, Canada, and the United States. NAPPO's Biotechnology Panel advises NAPPO on biotechnology issues as they relate to plant protection, and NAPPO has developed a standard for the *Importation and Release into the Environment of Transgenic Plants in NAPPO Member Countries* (see http://www.nappo.org/Standards/Std-e.html.). APHIS also participates regularly in biotechnology policy discussions at forums sponsored by the European Union and the Organization for Economic Cooperation and Development. APHIS periodically holds discussions on biotechnology regulatory issues with other countries (e.g. with Canada, Mexico, Argentina, Brazil, Japan, China, Korea to name a few), and has participated in numerous conferences intended to enhance international cooperation on safety in biotechnology. APHIS has sponsored several workshops on safeguards for planned introductions of transgenic crops most of which have included consideration of international biosafety issues. Mexico and Brazil, both of which have relatives of cotton that can potentially interbreed with it, have procedures in place that require a full evaluation of transgenic plants before they can be introduced into the environment and both countries have ratified the Cartegena Protocol. Many countries, including Australia, Mexico, South Africa, China, and Argentina are already growing other approved varieties of Bt cotton (Carpenter et al., 2002). APHIS does not expect a significant environmental impact outside the United States should nonregulated status be granted for the subject Cotton Events Cry1F and Cry1Ac.

C. Alternative C, Approval of the Petition in Part

1. Approval of some, but not all, of the lines requested in a petition. Under this alternative, APHIS may consider approval of some, but not all, of the lines requested in a petition. Of the subject petitions, 03-036-01p requested a determination of nonregulated status for only one transformation event, Cotton Event Cry1F and progeny derived from this event by traditional breeding practices, and 03-036-02p requested a determination of nonregulated status for only one transformation event, Cotton Event Cry1Ac and progeny derived from this event by traditional breeding practices. Therefore, APHIS can consider only these transformation events for approval.

2. Approval of the petition with geographic restrictions. EPA is currently reviewing the application to register the use of the stacked cotton product derived from a cross between Cotton Events Cry1F and Cry1Ac under its regulations for plant-incorporated protectants. EPA has the

authority to impose geographic limitations on the use of specific pesticides and routinely does so to protect threatened and endangered species, as well as other non-target organisms. EPA and APHIS agree that the threatened and endangered lepidopteran species do not typically feed on cotton, so they are not likely to be exposed to the Cry1F (synpro) and Cry1Ac (synpro) proteins expressed in the stacked product. Cotton plants are not considered to be wind pollinated, so it is not likely that the relatively heavy pollen grains will move from the cotton plants to rest on the surface of other substrates that will be ingested by these threatened and endangered lepidopteran species. On the basis of these considerations, APHIS can find no reason for placing geographic restrictions on planting of Cotton Events Cry1F and Cry1Ac or their stacked product by granting the petition in part.

VI. LITERATURE CITED

Baker, H.G. 1965. Characteristics and Modes of Origin of Weeds. *In:* Baker, H.G., Stebbin, G.L. (eds.) The Genetics of Colonizing Species. pp. 147-172. Academic Press, Academic Press, New York and London.

Baker, H.G. 1974. The evolution of weeds. Annual Review of Ecology and Systematics 5: 1-24.

Carpenter, J., Felsot, A., Goode, T., Hammig, M., Onstad, D., Sankula, S. 2002. Comparative Environmental Impacts of Biotechnology-derived and Traditional Soybean, Corn, and Cotton Crops. The Council for Agricultural Science and Technology, Ames, Iowas. www.cast-science.org. Sponsored by the United Soybean Board.

Carrière, Y., Ellers-Kirk, C., Sisterson, M., Antilla, L., Whitlow, M., Dennehey, T.J., and Tabashnik, B.E. 2003. Long-term regional suppression of pink bollworm by *Bacillus thuringiensis* cotton. Proc. Natl. Acad. Aci. USA 100: 1510-1523.

Fernandez-Cornejo, J. and McBride, W.D. 2002. Adoption of bioengineered crops. Economic Research Service, USDA. Agriculture Economic Report No. 810 (AER-810).

Fish and Wildlife Service. 2001. Endangered and Threatened Wildlife and Plants; Proposed Determination of Critical Habitat for the Quino Checkerspot Butterfly; Proposed Rule. Federal Register. 66 (26) 9475-9507.

Fish and Wildlife Service. 1994. Endangered and Threatened Wildlife and Plants; Proposed Rule to List the Saint Francis' Satyr as Endangered. Federal Register 59, 443 18350 (04/18/94) 443

Fish and Wildlife Service. 1997. Endangered and Threatened Wildlife and Plants; Determinatiun of Endangered Status of the Laguna Mountains Skipper and Quino Checkerspot Butterfly. Final rule. Federal Register. 62 (11) 2313-2322).

Fryxell, Pl.A. 1984. Cotton. Pp. 27-57. In, Kohel, R.J. and Lewis, C.F., Ed., Taxonomy and Germplasm Resources. American Soc. Agron., Crop Sci. Soc. Of Amer. And Soil Sci. Soc. Of Amer. Madison, Wi.

Fryxell, P.A. 1979. The Natural History of the Cotton Tribe (Malvaceae, Tribe Gossypieae). Texas A&M University Press, College Station, TX.

Gianessi, L.P., Carpenter, J.E. 1999. Agricultural Biotechnology: Insect Control Benefits. http://www.ncfap.org/pubs.htm#Biotechnology.

Godfrey, L, Goodell, P. Grafton-Cardwell, E., Toscano, N. Natwick, E.T. Brazzel, J. 2000. UC IPM Pest Management Guidelines: Cotton. The Regents of the University of California. (http://www.ipm.ucdavis.edu/PMG/r114303511.html.)

Hardee, D.D., van Duyn, J.W., Layton, M.B. and Bagwell, R.D. 2001. Bt Cotton and management of the tobacco budworm-bollworm complex. USDA ARS. Agricultural Research Service Publication ARS-154. 40 p.

Hellmich, R.L., Siegfried, B.D., Sears, M.K., Stanley-Horn, D.E., Daniels, M.J., Mattila, H.R., Spencer, T., Bidne, K.G., Lewis, L.C. 2001. Monarch larvae sensitivity to *Bacillus thuringiensis*-purified proteins and pollen. Proc. Nat. Acad. Sci. 98 (21): 11925-11930.

Hofmann, C., Luthy, P., Hutter, R., Piska, V. 1988a. Binding of the Delta Endotoxin from *Bacillus thuringiensis* to Brush-Border Membrane Vesicles of the Cabbage Butterfly (*Pieris brassicae*). Eur. J. Biochem. 173:85-91.

Hofmann, C., Vanderbruggen, H., Hofte, H., Van Rie, J., Jansens, S., Van Mellaert, H. 1988b. Specificity of *B. thuringiensis* Delta-Endotoxins is Correlated with the Presence of High Affinity Binding Sites in the Brush-Border Membrane of Target Insect Midguts. Proc. Natl. Acad. Sci. USA 85:7844-7448.

Holm, L.G., Plucknett, D.L., Pancho J.V., and Herberger, J.P. 1977. The World=s Worst Weeds: Distribution and Biology. University Press of Hawaii, Honolulu.

Holm, L.G., Pancho J.V., Herberger, J.P., and Plucknett, D.L. 1979. Geographical Atlas of World Weeds. John Wiley and Sons, NY.

Holm, L.G., Doll, J., Holm, E., Pancho J.V., and Herberger, J.P. 1997. World Weeds; Natural Histories and Distribution. John Wiley and Sons, NY.

Lambert, B., Buysse, L., Decock, C., Jansens, S., Piens, C., Saey, B., Seurinck, J., Van Audenhove, K., Van Rie, J., Van Vliet, A., Peferoen, M. 1996. A *Bacillus thuringiensis* insecticidal crystal protein with a high activity against members of the family Noctuidae. Appl. Envir. Microbiol. 62(1):80-86.

Lewis, P.I. 2002. Memorandum to Marcia E. Mulkey, Director, Office of Pesticide Programs. Subject: Transmittal of Meeting Minutes of the FIFRA Scientific Advisory Panel Meeting Held August 27-29, 2002. Available at http://www.epa.gov/oscpmont/sap/2002/august/august2002final.pdf.

Losey, J.E., Rayor, L.S., Carter, M.E. and Smith, M.E. 1999. Transgenic pollen harms monarch larvae. Nature 399, 214.

Muenscher, W. C. 1980. Weeds. Second Edition. Cornell University Press, Ithaca and London. 586 pp.

National Research Council 1989. Field Tessting Genetically Modified Organisms: Framework for Decisions. National Academy Press, Washington, D.C.

Reed, C.F. 1977. Economically important foreign weeds: potential problems in the United States. Washington, D.C. APHIS, USDA. Ag. Handbook No. 498. 746 pp.

Sears, M.K., Hellmich, R.L., Stanley-Horn, D.E., Oberhauser, K.S., Pleasants, J.M., Mattila, H.R., Siegfried, B.D., Dively, G.L. 2001. Impact of Bt corn pollen on monarch butterfly populations: A risk assessment. Proc. Nat. Acad. Sci. 98: 11937-11942

Shelton, A.M., Zhao, J.-Z., Roush, R.T. 2002. Economic, ecological, food safety, and social consequences of the deployment of Bt transgenic plants. Annu. Rev. Entomol. 47: 845-881.

Southern Weed Science Society. 1998. Weeds of the United States and Canada. CD-ROM. Southern Weed Science Society. Champaign, Illinois.

Smith, C.W. and Cothren, J.T. (editors). 1999. Cotton: origin, history, technology, and production. 850 p.

Umbeck, P. F., Barton, K. A., Nordheim, E, V., McCarty, J. C, Parrott, W. L., and Jenkins, J. N. 1991. Degree of Pollen Dispersal by Insects from a Field Test of Genetically Engineered Cotton. J. Econ. Entomology 84:1943-1991.

US EPA. 2001. Biopesticides Registration Action Document - *Bt* Plant-Incorporated Protectants. October 16, 2001 http://www.epa.gov/pesticides/biopesticides/pips/bt_brad.htm.

US EPA. 2001b. 40 CFR Parts 152 and 174 - Plant-Incorporated Protectants; Final Rules and Proposed Rule. Federal Register July 19, 2001. http://www.epa.gov/pesticides/biopesticides/pip/pip_rule.pdf.

US EPA. 2002. Biopesticide Fact Sheet. *Bacillus thuringiensis* Cry2Ab2 protein and the genetic material necessary for its production in cotton (006487). US EPA, Office of Pesticide Programs.

http://www.epa.gov/pesticides/biopesticides/ingredients/factsheets/factsheet_006487.htm.

USDA, NRCS. 2001. The PLANTS Database, Version 3.1 (http://plants.usda.gov). National Plant Data Center, Baton Rouge, LA 70874-4490 USA.

Van Rie, J. Jansens, S., Hofte, H., Degheele, D., Van Mellaert, H. 1989. Specificity of *Bacillus thruringiensis* δ-Endotoxins, Importance of Specific Receptors on the Brush Border Membrane of the Mid-Gut of Target Insects. Eur. J. Biochem. 186:239-247.

Van Rie, J. Jansens, S., Hofte, H., Degheele, D., Van Mellaert, H. 1990. Receptors on the Brush Border Membrane of the Insect MidGut as Determininants of the Specificity of *Bacillus thruringiensis* Delta-Endotoxins. Appl. Environ. Microbiol. 56:1378-1385.

Weed Science Society of America. 1989. Composite List of Weeds. WSSA. Champaign, Illinois.

Wolfersberger, M.G., Hofmann, C., Luthy, P. 1986. *In* Bacterial Protein Toxins. (eds. Falmagne, P., Alout, J.E., Fehrenbach, F.J., Jeljaszewics, J. and Thelestam, M.) pp. 237-238. Fischer, New York.

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Appendix A: Biology of cotton and potential for introgression into related species.

Cotton as a Crop

Four species of the genus *Gossypium* are known as cotton, which is grown primarily for the seed hairs that are made into textiles. Cotton is predominant as a textile fiber because the mature dry hairs twist in such a way that fine, strong threads can be spun from them. Other products, such as cottonseed oil, cake, and cotton linters are byproducts of fiber production.

Cotton, a perennial plant cultivated as an annual, is grown in the United States mostly in areas from Virginia southward and westward to California, in an area often referred to as the Cotton Belt (McGregor, 1976).

Taxonomy of Cotton

The genus *Gossypium*, a member of the Malvaceae family, consists of some 50 species, four of which are generally cultivated (Fryxell, 1992). The most commonly cultivated species, *G. hirsutum* L., is the subject of this Environmental Assessement. Other cultivated species are *G. arboreum* L., *G. barbadense* L., and *G. herbaceum* L.

Four species of Gossypium occur in the United States (Fryxell, 1979; Kartesz and Kartesz, 1980). *Gossypium hirsutum* is the primary cultivated cotton. *Gossypium barbadense* is also cultivated. The other two species, *G. thurberi* Todaro and *G. tomentosum* Nuttall ex Seemann, are wild plants of Arizona and Hawaii, respectively. *Gossypium tomentosum* is known from a few strand locations very close to the ocean.

Genetics of Cotton

At least eight genome designations, A, B, C, D, E, F, G and K, are found in the genus (Endrizzi *et al.*, 1985). Diploid species (2n=26) are found on all continents, and a few are of some agricultural importance. The A genome is restricted in diploids to two species (*G. arboreum*, and *G. herbaceum*) of the Old World. The D genome is restricted in diploids to some species of the New World, such as *G. thurberi*.

By far, the most important agricultural cottons are *G. hirsutum* and *G. barbadense*. These are both allotetraploids of New World origin, and presumably of ancient cross between Old World A genomes and New World D genomes. How and when the original crosses occurred have been subject to much speculation. Euploids of these plants have 52 somatic chromosomes, and are frequently designated as AADD. Four additional New World allotetraploids occur in the genus, including *G. tomentosum*, the native of Hawaii. *Gossypium tomentosum* has been crossed with *G. hirsutum* in breeding programs.

The New World allotetraploids are peculiar in the genus, because the species, at least in their wild forms, grow near the ocean, as invaders in the constantly disturbed habitats of strand and associated

environs. It is from these "weedy" or invader species that the cultivated cottons developed (Fryxell, 1979).

Weediness of Cotton

Although the New World allotetraploids show some tendencies to "weediness" (Fryxell, 1979), the genus shows no particular weedy aggressive tendencies.

Pollination of Cotton

Gossypium hirsutum is generally self-pollinating, but in the presence of suitable insect pollinators can exhibit cross pollination. Bumble bees (*Bombus* spp.), Melissodes bees, and honey bees (*Apis mellifera*) are the primary pollinators (McGregor, 1976). Concentration of suitable pollinators varies from location to location and by season, and is considerably suppressed by insecticide use. If suitable bee pollinators are present, distribution of pollen decreases considerably with increasing distance. McGregor (1976) reported results from an experiment in which a cotton field was surrounded by a large number of honey bee colonies, and movement of pollen was traced by means of fluorescent particles. At 150 to 200 feet, 1.6 percent of the flowers showed the presence of the particles. The isolation distance for Foundation, Registered, and Certified seed in 7 CFR Part 201 is 1320 feet, 1320 feet, and 660 feet, respectively.

Research in Mississippi shows that pollen movement decreases rapidly after 40 feet (12 meters). Umbeck et al. (1991) studied pollen and successful gene movement of cotton in Mississippi test plots. Around a central transgenic test plot of 98,800 plants with rows running north-south, they planted 23 one-meter border rows of nontransgenic cotton to the east and to the west, and 25 meters of non transgenic cotton border rows to the north and to the south, each divided into two 12.5 meter long plots. The border rows to the north and south were continuous with the transgenic rows. They took 32,187 seed samples from all border rows at bottom, middle, and top plant position (representing seasonal variation) and used a kanamycin resistance marker gene to test for seeds resulting from pollen movement out of the central transgenic plot. To the east and west, gene movement at the first row was 0.057 and 0.050, and dropped rapidly to row 8, and was not detected in subsequent rows to the east, and detected occasionally at <0.01 in rows to the west. Combined data for east and west border rows beyond row 9 gave total outcrossing of 0.0012. To the north and south, detections were totaled for each 12.5 meter block and gave figures of 0.0053 and 0.0047 for north and south inner block and 0.0015 and 0.0021 for north and south outer block.

Modes of Gene Escape in Cotton

Genetic material of *G. hirsutum* may escape from an area of cultivation by vegetative material, by seed, or by pollen. Propagation by vegetative material is not a common method of reproduction of cotton. Movement of seed can occur on farm implements such as planters and harvesters and can be minimized by cleaning of equipment between plots when separation of crop varieties is desired.

Movement of genetic material by pollen is possible only to those plants with the proper chromosomal type, in this instance only to those allotetraploids with AADD genomes. In the United States, this would only include *G. hirsutum*, *G. barbadense*, and *G. tomentosum*. *Gossypium thurberi*, the native diploid from Arizona with a DD genome, is not a suitable recipient. Movement to *G. hirsutum* and *G. barbadense* is possible if suitable insect pollinators are present, and if there is a short distance from transgenic plants to recipient plants. Physical barriers, intermediate pollinator-attractive plants, and other temporal or biological impediments would reduce the potential for pollen movement.

Movement of genetic material to *G. tomentosum* is less understood. The plants are chromosomally compatible with *G. hirsutum*, but there is some doubt as to the possibility for pollination. The stigma in *G. tomentosum* is elongated, and the plant seems incapable of self-pollination until acted upon by an insect pollinator, but flowers of *G. tomentosum* seem to be pollinated by moths, not bees. And they are receptive at night, not in the day. Most *Gossypium* flowers are ephemeral: they open in the morning and wither at the end of the same day. Both these factors would seem to minimize the possibility of cross-pollination. However, Fryxell (1979) reports that *G. tomentosum* may be losing its genetic identity from introgression hybridization of cultivated cottons by unknown means.

LITERATURE CITED

Endrizzi, J. E., Turcotte, E. L., and Kohel, R. J. 1985. Genetics, cytogenetics, and evolution of *Gossypium*. Advances in Genetics 23: 271-375.

Fryxell, P. A. 1979. The Natural History of the Cotton Tribe (Malvaceae, Tribe Gossypieae). Texas A&M University Press. College Station and London. 245 pp.

Fryxell, P. A. 1984. Taxonomy and Germplasm Resources. pp. 27-57. In Kohel, R. J. and Lewis, C. F., Editors. Cotton. American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America. Madison, Wisconsin. 605 pp.

Fryxell, P. A. 1992. A revised taxonomic interpretation of *Gossypium* L. (Malvaceae). Rheedea 2:108-165.

Umbeck, P. F., Barton, K. A., Nordheim, E, V., McCarty, J. C, Parrott, W. L., and Jenkins, J. N. 1991. Degree of Pollen Dispersal by Insects from a Field Test of Genetically Engineered Cotton. J. Econ. Entomology 84:1943-1991.

Kartesz, J. T., and Kartesz, R. 1980. A Synonymized Checklist of the Vascular Flora of the United States, Canada, and Greenland. The University of North Carolina Press. Chapel Hill.

McGregor, S. E. 1976. Insect Pollination of Cultivated Crop Plants. Agriculture Handbook No. 496. U.S. Government Printing Office. Washington, DC.

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Appendix B. List of APHIS authorized field tests of Cotton Event Cry1F, Cotton Event Cry1Ac, and the Stacked Product Cry1F/Cry1Ac. (Derived from Mycogen/Dow field data reports for authorizations listed in 03-036-01p and 03-036-02p and information supplied on Feb. 19, 2003.)

MS*	USDA#	States:Counties of Release	Cotton Events			Year Planted	Total Acres Planted
			Cry1F	Cry1Ac	Stack		
MS266	02-302-12n	Not yet planted	X	X	X		
MS259	02-249-02n	Not yet planted	X	X	X		
MS234	02-066-09n	**MS**:Washington; **SC**:Darlington; **TX**:Haskell	X X X	X X X	X X X	2002	<0.7 acres
MS232	02-066-07n	**AL**:Baldwin; **AR**:Drew, Jackson, Woofruff; **AZ**:Pinal; **CA**:Fresno, **GA**:Decatur; **LA**:ST. Landry, Rapides, Franklin; **MS**:Oktibbeha, Washington, **NC**:Washington, Martin; **NM**:Dona Ana: **SC**:Barnwell; **TN**:Shelby; **TX**:SanPatricio, Waller, Burleson; **AR**: Lonoke, Phillips; **CA**:Kings; **MS**:Webster; **TN**:Haywood	X X X X X X X X X X X X X X X X X X	X X X X X X X X X X X X X X X X X	X X X X X X X X X X X X X X X X X	2002	26.6 acres.
MS231	02-066-06n	**AZ**:Pinal; **GA**:Mitchell; **MS**:Washington; **TN**:Shelby; **TX**:Lubbock	X X X X X	X X X X X	X X X X X	2002	0.8 acres
MS198	01-093-17n	**AZ**:Pinal; **CA**:Fresno; **MS**:Washington; **TX**:Armstrong, Wharton; **VA**:Gates	X X X X X X	X X X X X X	X X X X X X	2001	~1.5 acres
MS196	01-093-14n	**AZ**:Pinal; **MS**:Bolivar; **SC**:Darlington	 X X	 X X	X X X	2001	<0.6 acres

Appendix B. Continued…

MS*	USDA#	States:Counties of Release	Cotton Events Cry1F	Cry1Ac	Stack	Year Planted	Total Acres Planted
MS185	01-052-10n	**AZ**:Pinal; **GA**:Mitchell; **MS**:Washington; **TN**:Shelby; **TX**:Lubbock	X X X X X	X X X X X	X X X X X	2001	<0.6 acres
MS181	01-052-06n	**AL**:Baldwin; **AZ**:Pinal; **CA**:Kings, Fresno; **LA**:Franklin; **MS**:Washington (3), Oktibbeha; **NC**:Martin; **PR**:Santa Isabel	X X X X X X X X	X X X X X X X X	X X X X X X X X	2001	<9.0 acres
MS163	00-265-07n	**PR**:Santa Isabel	X			2000-01	0.7 acres
MS145	00-111-11n	**AL**:Macon	X			2000	0.1 acres
MS105	00-049-15n	**MS**:Washington, Oktibbeha; **CA**:Kings; **NC**:Martin; **CA**:Fresno; **GA**:Tift; **LA**:Franklin	X X X X X X X	X X X X		2000	<2.6 acres
MS100	00-047-07n	**AZ:**Pinal	X			2000	<1.0 acre
MS077	99-067-09n	**MS:** Oktibbeha, Washington		X X		1999	<1.9 acres

MS183: 01-052-08n field data report indicates that the Cotton Event Cry1F and Cry1Ac were not planted in this trial, only a cross between Cotton Event Cry1F and a different Cry1Ac cotton transformation event. The inclusion of this notification in the Appendix 1. for both petitions is an error.

Appendix C. Table comparing environmental fate and effects of Cry1F and Cry1Ac expressed in Mycogen/Dow Cotton Events with other insecticides used to control lepidopteran pests of cotton in the United States.

Mycogen/Dow Cotton Event 3006-210-23 contains the Cry1Ac (synpro) protein and Event 281-24-236 contains Cry1F (synpro) protein. Cotton line MXB-13 is the end-use stacked product from a cross between Event 3006-210-23 and Event 281-24-236. The proposed label for the seed of the stacked product with both Cry1F and Cry1A(c) indicates that it is for control against cotton bollworm (*Helicoverpa zea*), pink bollworm (*Pectinophora gossypiella*), and tobacco budworm (*Helothis virescens*), beet armyworm (*Spodoptera exigua* H.), fall armyworm (*Spodoptera frugiperda* S.), southern armyworm (*Spodoptera eridania* S.), soybean looper (*Pseudoplusia includens* W.) and cabbage looper (*Trichoplusia ni* H.) (personal communication with Irene Gatti, Dow, on Jan. 28, 2004). The EPA has not yet approved this label.

Bollgard I and II are effective against the cotton bollworm, tobacco budworm and pink bollworm. Bollgard II is also effective against beet armyworm, fall armyworm, southern armyworm, soybean looper, cabbage looper, saltmarsh caterpillar (*Estigmene acrea*), cotton leaf perforator (*Bucculatrix thurberiella*), yellowstriped armyworm (*Spodoptera ornithogalli*), and European corn borer (*Ostrinia nubilalis*). Growers report that Bollgard I is effective against budworm, but additional measures to control bollworm may be necessary when infestation levels are high.

The Cry proteins in these Bt cottons are derived from various *Bacillus thuringiensis* spore-forming gram positive bacterium. These bacterica are naturally occurring in soils worldwide at significant levels. They have been used commercially for nearly 40 years to control insects. The Cry proteins in these products have an established history of environmental safety, as summarized in the EPA 2001 Regulatory Eligibility Decision Document.

Pyrethroids are used commonly for bollworm control, and are also routinely used to control boll weevil, as well as cutworm, cabbage looper, and stink bugs. Pyrethroids are routinely used in combination with Bollgard I, particularly when bollworm levels are high. Pyrethroids are not recommended for budworm control because of resistance.

Organophosphates are used to control budworm and bollworm, as well as a number of secondary pests in cotton. Methyl parathion is effective against boll weevil as well. Malathion is used routinely for control of boll weevil, but is not effective against budworm or bollworm.

The carbamates can be effective against budworm and bollworm, but are used more commonly to control cabbage and soybean looper, and fall and beet armyworm. Emamectin benzoate has been approved for use in a number of states under a section 18 emergency exemption to control resistant tobacco budworm and severe infestations of beet armyworm.

Spinosad and Indoxacarb are used routinely to control tobacco budworm. These insecticides are also effective against cotton bollworm, cabbage looper, soybean looper, fall armyworm, beet armyworm, and cutworm, but they are not effective against boll weevil.

	Cotton Events - Cry1A(c) (3006-210-23) Cry1F (281-24-236) and the Stacked product Cry1Ac/Cry1F (MXB-13)	Bollgard I® – Cry1Ac (Lines 531, 757, 1076) Bollgard II® – Cry2Ab (Event 15985)	Cyfluthrin (Baythroid) Lambda Cyhalothrin (Karate Z) Bifenthrin (Capture)	Malathion (Fyfanon) Profenofos (Curacron) Methyl Parathion	Spinosad (Tracer)
	Bt Cotton	Bt Cotton	Pyrethroids	Organophosphates	Naturalyte
Expression Levels of Cry proteins and Application Rate or Formulation of Chemical Insecticides (when available from sources consulted)	High end exposure estimates (HEEE) in ng/mg tissue (unless otherwise stated): Cry1Ac in Event 3006-210-23 & MXB-13, respectively are 3.29 and 2.09 in terminal leaves, 2.18 and 0.99 in seedling whole plants, 0.40 and 0.19 in roots at defoliation, 2.42 and 2.43 in pollen, 0.75 and 0.69 in seeds, and in nectar it is <0.05 ng/µL. Cry1F protein in Event 281-24-236 and MXB-13, respectively, are 18.1 and 15.1 in terminal leaves, 40.5 and 43.6 in the whole plant at defoliation, 1.6 and 0.9 in roots at defoliation, 0.7 and 0.4 in pollen, 7.5 and 6.3 in seeds, and <0.05 ng/µL in nectar.	Cry1Ac expression levels in cotton have been determined. Cotton lines 531 (Bollgard I) and 931 were used. The Cry protein is detectable in leaves (1.1-2.04 g/g), seeds (0.49-1.62g/g), and whole plant assays; undetectable in cottonseed meal; at or near the level of detection in pollen (11.5ng/g); below the level of detection in nectar (<1.6ng/g); and undetectable in refined oil. Cry2Ab2 expression levels in Bollgard II cotton lines 15813 and 15985 averaged 11.3 - 26.8 µg/fresh wgt leaves (HEE at 55 days post planting 40.1 ± 6.5); 37 - 43.2 µg/fresh wgt seed; <0.25 - 1.7 µg/fresh wgt pollen; and 4.15 – 8.8 in the whole plant	**Cyfluthrin** formulations are available in the form of emulsifiable concentrates, wettable powder, aerosol, granules, liquid, oil-in-water emulsion and ULV oilspray. 0.0125 - 0.05 pounds/acre are typically applied for agricultural uses. **Lambda Cyhalothrin** is available as an emulsifiable concentrate, wettable powder or ULV liquid and is commonly mixed with buprofezin, pirimicarb, dimethoate or tetramethrin . It is compatible with most other insecticides and fungicides **Bifenthrin** available as an emulsifiable concentrate or a wettable powder	**Malathion** is available in emulsifiable concentrate, wettable powder, dustable powder, and ULV liquid formulations. Malathion may also be found in formulations with many other pesticides. **Profenofos** is registered for cotton use only. It is applied as a foliar spray at a rate of up to 1.0 lb a.i./acre. It should be applied from planting through defoliation with a maximum of six applications per season. **Methyl Parathion** is available in dust, emulsifiable concentrate, ULV liquid, and wettable powder formulations	**Spinosad** is registered for cotton use as a foliar application. Application rates are 0.04 to 0.09 lbs a.i/acre per application and up to 0.45 lbs a.i./acre each growing season.
Environmental Fate	The Estimated Environmental Concentrations (EEC) for the single Cotton Events and stacked product, respectively are as follows: Cry1Ac in soil, based on average cotton yields of 1.35 bales per acre are 0.0349 and 0.0196 mg a.i./kg soil. The EEC for Cry1Ac in surface water due to runoff/erosion to an edge of field pond in the two days immediately post-incorporation from Cry1Ac returned to the soil to a depth of six inches was 191 and 107 ng/L. The EEC's for Cry1F in soil are	Data produced by Monsanto for Cry1Ac in cotton give degradation rates (DT_{50}) of approximately 9-20 days for the purified protein, and 41 days for the protein in cotton tissue. This study demonstrated a loss, following soil incorporation, in activity of *Btk* endotoxin against tobacco budworm. Data also indicate that Cry protein production ceases at senescence in cotton, allowing time for protein degradation before harvest. Furthermore, environmental fate data indicate that only a small amount of Cry	**Cyfluthrin** is sensitive to breakdown by sunlight. On the surface of soils, its half-life is 48-72 hours. It has a half-life of 56-63 days in German loam and sandy loam soils, respectively. Cyfluthrin is very immobile in soils, and is not considered a threat to contaminate groundwater. Cyfluthrin is broken down quickly in surface water. Because it is relatively non-soluble, and less dense than water, it will float on the surface film of natural waters. At the surface, it is subject to breakdown by exposure to sunlight (1 day). There is little	**Malathion** is of low persistence in soil with reported field half-lives of 1 to 25 days. Degradation in soil is rapid and related to the degree of soil binding. It is moderately bound to soils, and is soluble in water, so it may pose a risk of groundwater or surface water contamination in situations which may be less conducive to breakdown. In raw river water, the half-life is less than 1 week, whereas malathion remained stable in distilled water for 3 weeks. Residues were found mainly associated with areas of high lipid content in plants.	**Spinosad** is relatively short-lived in the field and photodegrades rapidly. Data show that Spinosad and its aged residues are unlikely to leach in most soils, are relatively immobile, and pose little threat to groundwater. The metabolism in cotton as a result of foliar application of spinosad is adequately understood. No trace was found in various cotton seed fractions. Based on field trials, residues of spinosad are not expected to be detectable in cottonseed at 0.01ppm, the limit of quantification. Concentration

	Cotton Events - Cry1A(c) (3006-210-23) Cry1F (281-24-236) and the Stacked product Cry1Ac/Cry1F (MXB-13)	Bollgard I® – Cry1Ac (Lines 531, 757, 1076) Bollgard II® – Cry2Ab (Event 15985)	Cyfluthrin (Baythroid) Lambda Cyhalothrin (Karate Z) Bifenthrin (Capture)	Malathion (Fyfanon) Profenofos (Curacron) Methyl Parathion	Spinosad (Tracer)
	0.324 and 0.317, and in water are 1,290 and 1,710 ng/L. Based on bioassay results measuring 50% growth inhibition of sensitive insects (GI_{50}s) for soil amended with lyophilized MXB-13 cotton tissue, the DT_{50} (time to 50% degradation) of the Cry1F/Cry1Ac proteins was 1.3 days under laboratory conditions, indicating a rapid decay rate in soil. Studies have shown that out-crossing frequency from transgenic cotton pollen declined from 0.61% at 5 m off-source to 0.03% at 50m and was not detectable at 100m, thus the occurrence of cotton as contaminants on host plants will be negligible. Studies have also shown that the concentration of transgenic protein found in foliar feeding herbivores clearly showed the reduction in protein that can be expected in a multitrophic context.	protein (~1.44g per acre) enters the soil following post harvest incorporation of Bt cotton, and such proteins degrade rapidly, such that the potential for effects on non-target soil organisms is not anticipated Cry2Ab2 + Cry1Ac proteins degrade rapidly in sandy loam soil typical for cotton production. The DT_{50} was 2.3 days, DT_{90} was 15 days for Bollgard II, and 75% of the protein degrades in the first week. However, this study used the cotton bollworm (*Helicoverpa zea*) as the indicator species in the bioassay, which is not not as sensitive to Cry2Ab2 as other lepidopterans and it is less sensitive to Cry2Ab2 than Cry1Ac. However, the presence of Cry1Ac was not considered in the data analysis. Therefore an accurate degradation time (DT_{50}) was not determined since there is not a high dose of Cry2Ab2 or Cry1Ac expressed to control the cotton bollworm.	information available about the breakdown of cyfluthrin in vegetation. **Lambda Cyhalothrin** is moderately persistent in the soil environment. Its field half-life is probably close to 30 days in most soils. It shows a high affinity for soil. It is not appreciably mobile in most soils and there is little potential for groundwater contamination. Lambda cyhalothrin has extremely low water solubility. No data were available regarding the breakdown of lambda cyhalothrin in vegetation. **Bifenthrin** has low mobility in most soils and is relatively insoluble in water, so there are no concerns about groundwater contamination through leaching. Its half-life in soil is 7 days to 8 months depending on the soil type and the amount of air in the soil. It is not absorbed by plant foliage, nor does it translocate in the plant.	**Profenofos** has a half-life of 1.9 days in average soils, and 23-62 days in water. Leaching potential is slight and soil mobility is low. **Methyl Parathion** has low soil persistence, with reported field half-lives of 1 to 30 days. A representative value is estimated to be 5 days. Degradation increases with temperature and with exposure to sunlight. Methyl parathion is moderately adsorbed by most soils, and is slightly soluble in water. Due to its low residence time and soil binding affinity, it is not expected to be significantly mobile. Some volatilization of applied methyl parathion may occur. It degrades rapidly in seawater, lake, and river waters, with 100% degradation occurring within 2 weeks to 1 month or more. Its uptake and metabolism in plants is fairly rapid. Within 4 days of application to corn, it was almost completely metabolized.	of residues in cottonseed process fractions at 6x maximum label rate were not found. There are no acute chronic levels of concern (LOC) exceeded for birds, terrestrial and freshwater aquatic organisms, or estuarine organisms.
Avian toxicity	An 8-day acute avian dietary study with bobwhite quail testing the effect of a 10% cotton meal diet using meal prepared from cottonseed expressing 0.021 µg/g Cry1F and 0.012 µg/g Cry1Ac proteins. There were no adverse effects of treatment. The LC_{50} was >100,000 µg meal/g diet and >2100 ng Cry1F or >1200 ng Cry1Ac per	Ground cottonseed expressing 0.9 ng Cry1Ac *Btk* protein/g fresh wt showed no toxicity to northern bobwhite quail when fed at 10,000 ppm in the diet for 5 days. Bobwhite quail fed cottonseed expressing cry2Ab2 (Bollgard II) at 10% of the diet exhibited no adverse effects. Ground cottonseed expressing 100,000	The toxicity of these pyrethroid insecticides to birds range from practically non-toxic to moderately toxic. **Cyfluthrin** is of low toxicity to upland game birds and waterfowl. LD_{50} values range from >2,000 mg/kg in acute oral tests with bobwhite quail, to >5,000 mg/kg in subacute tests with both mallard ducks	Malathion and Profenofos are moderately toxic to birds. Methyl Parathion is very highly to highly toxic to birds. **Malathion's** reported acute oral LD50 values are: in mallards, 1485 mg/kg; in pheasants, 167 mg/kg; in blackbirds and starlings, over 100 mg/kg; and in chickens, 525 mg/kg The reported 5- to 8-day dietary	**Spinosad** is practically nontoxic to slightly toxic to northern bobwhite quail and mallard duck. The acute oral LD_{50} is >1333 mg/kg. The acute dietary LC_{50} is >5156ppm.

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	gram diet. When Cry1F and Cry1Ac were administered in combination as a single oral dose, no mortality occurred and the no-observed-effect level was 113.6 mg/kg for Cry1F and 14.4mg/kg for Cry1Ac.	ppm Cry1Ac protein/g fresh wt showed no toxicity to northern bobwhite quail when fed at 10,000 ppm in the diet for 5 days. The dietary LC_{50} for Cry2Ab2 cottonseed meal fed to catfish was greater than 20% of diet which was the highest dose tested. No behavior change was observed between catfish fed with Cry2Ab2 and those fed cottonseed meal from non-genetically modified cotton.	and bobwhite quail. **Lambda Cyhalothrin's** toxicity to birds ranges from slightly toxic to practically non-toxic. In the mallard duck the reported dietary LC_{50} is >3,948 and in bobwhite quail the LC_{50} is >500ppm. There is evidence that it does not accumulate in the eggs or tissues of birds. **Bifenthrin** is moderately toxic to many species of birds. The 8-day LC_{50} is 1,280 ppm for mallard ducks and 4,450 ppm for bobwhite quail. The acute oral LD_{50} is 1,800 mg/kg for bobwhite quail and 2,150 mg/kg for mallard ducks. There is concern about possible bioaccumulation in birds.	LC_{50} is >3000 ppm in mallard and northern bobwhite. **Profenofos** acute toxicity studies showed moderate toxicity using a single-dose oral study on mallard ducks. An 8-day dietary study on bobwhite quail indicated an LC_{50}/EC_{50} of 57 ppm, and on mallard ducks 1647 ppm. A chronic toxicity study for avian species showed significant effects on egg production due to parental toxicity. **Methyl Parathion** reported acute oral LD_{50} values are 6 to 10 mg/kg in mallards and 8 mg/kg in northern bobwhites. The 5- to 8-day dietary LC_{50} values 330 to 680 ppm in mallard and 90 ppm in northern bobwhite.	
Aquatics toxicity	The acute dietary toxicity of Cry1F and Cry1Ac protein to rainbow trout (*Onchorynchus mykiss*) was determined for fish exposed for 8-days to a diet containing 10% cotton meal prepared from cotton seed expressing both proteins. This produced a diet containing 0.209 µg Cry1F and 0.118 µg Cry1Ac per gram food. No fish mortality or sublethal effects were observed. The LC_{50} is >0.209 mg Cry1F/kg diet, representing 162x the anticipated EEC in water, and is >0.118 mg Cry1Ac/kg diet, representing 618x the anticipated EEC. There are no known adverse	There is no evidence for sensitivity of aquatic species to Cry1Ac or Cry2Ab2. EPA waived the requirements for aquatic organism testing for Cry1Ac because of a lack of exposure. Only limited amounts of pollen would be available for exposure to aquatic invertebrates through drift. There have been no reports of hazard from feeding of cottonseed meal to farm fish. Channel catfish fed cottonseed expressing Cry2Ab (Bollgard II) at 20% of the diet exhibited no mortality and no adverse effects on survival, growth, or behavior.	These pyrethroid insecticides are highly to very highly toxic to aquatic organisms. **Cyfluthrin** is highly toxic to marine and freshwater organisms. The LC_{50} of cyfluthrin in water was 0.00068 mg/Lin rainbow trout. Cyfluthrin is exceptionally toxic to the freshwater invertebrate *Daphnia magna*, (LC_{50} = 0.14 ng/L or .00000014 mg/L). Marine and estuarine invertebrates are also extremely sensitive to cyfluthrin. **Lambda Cyhalothrin** is very highly toxic to many fish and aquatic invertebrate species. The reported LC_{50} in rainbow	The organophosphates range in their toxicity to aquatic organisms. **Malathion** has a wide range of toxicities in fish, extending from very highly toxic in the walleye (96-hour LC_{50} of 0.06 mg/L) to highly toxic in brown trout (0.1 mg/L) , moderately toxic in fathead minnows (8.6 mg/L) and slightly toxic in goldfish (10.7 mg/L). Various aquatic invertebrates are extremely sensitive, with EC_{50} values from 1 µg/L to 1 mg/L. Malathion is highly toxic to aquatic invertebrates and to the aquatic stages of amphibians. **Profenofos** laboratory data	**Spinosad** is slightly to moderately toxic to freshwater fish. The acute 96 hour LC_{50} for rainbow trout is 30ppm and for bluegill is 5.94ppm. Spinosad is moderately to highly toxic to estuarine organisms. The acute 96 hour LC_{50} for sheepshead minnows is 7.5ppm and for grass shrimp is >9.76ppm. The EC_{50} for eastern oyster is 0.3ppm. Spinosad is slightly toxic to *Daphnia*, with an acute 48 hour EC_{50} of 14ppm.

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	effects of Cry proteins on the aquatic invertebrate *Daphnia magna*. A test conducted with a combination of 2500 µg/L Cry1Ac (>13000x the EEC for Event 3006-210-23) and 510 µg/L Cry1F (395x the EEC for Event 281-24-236) resulted in no observable effects. The 24- and 48-hour EC_{50} for Cry1Ac is>2500 µg/L and for Cry1F is >510 µg/L.		trout is 0.24 µg/L, and in *Daphnia magna* is 0.36 µg/L. An EC_{50} for the eastern oyster of 0.59 ng/L has been reported. **Bifenthrin** is very highly toxic to fish, crustaceans and aquatic animals. The LC_{50} after a 96-hour exposure is 0.00015 mg/L for rainbow trout, and 0.0016 mg/L for Daphnia.	showed that profenofos is highly toxic to fish and aquatic invertebrates. The LC_{50}/EC_{50} for trout was 0.025 ppm, bluegill was 0.3 ppm, and daphnids was 0.0014 ppm. **Methyl Parathion** is moderately toxic to fish and to animals that eat fish. Reported 96-hour LC_{50} values are from 1.9 to 8.9 mg/L in a number of species tested. Reported 96-hour LC_{50} values indicate very high toxicity for aquatic invertebrates such as *Daphnia*.	
Honey bee toxicity	There were no effects on mean larval survival to adult emergence for honey bees exposed to either 2 mg cotton pollen from a Cry1Ac- or Cry1F- expressing event, or to 11.94 ug/mL of Cry1Ac and 1.98 ug/mLCry1F protein in combination. The LC_{50} for exposure to Cry1Ac protein is >11.94 µg/mL (5x the HEEE in Event 3006-210-23 pollen) and to Cry1F protein is >1.98 µg per mL (3x the HEEE in Event 281-24-236 pollen).	No adverse effects were observed in studies on honey bee larvae and adults at or above the maximum EEC of Cry1Ac or Cry2Ab in cotton to which honey bee is likely to be exposed. The LC50 of purified Cry1Ac Btk HD-73 crystals to adult and larval honey bees was 10,000 and 1,700 times the amount found in nectar and pollen, respectively. LC_{50} for purified cotton Cry2Ab2 protein fed to honey bee larvae is >100µg/mL (ppm).	These pyrethroid insecticides are highly toxic to bees. **Cyfluthrin** is highly toxic to bees with an LD_{50} of 0.037 mg/bee. **Lambda Cyhalothrin** is highly toxic to bees, with a reported oral LD_{50} of 38 ng/bee and reported contact LD_{50} of 909 ng/bee (0.9 µg/bee). **Bifenthrin** data for bees was not found in the sources consulted.	These organophosphates are toxic to bees. **Malathion** data on bees was not available in the sources consulted. **Profenofos:** The LC_{50}/EC_{50} for bees was 0.1 µg/bee. **Methyl Parathion** data on bees was not available in the sources consulted.	Laboratory data indicate **Spinosad** is highly toxic to honeybees. The 48 hour acute contact LD_{50} is 0.0029µg/bee.
Beneficial invertebrate toxicity	Dietary toxicity studies ~~to~~ conducted with microbe-derived Cry1Ac and Cry1F proteins, alone and in combination showed no toxicity to non-target invetebrates when fed levels many times greater than the levels found in pollen and nectar of Events 3006-210-23 and 281-24-236,	Susceptibility studies to Cry1Ac and Cry2Ab proteins were conducted on green lacewing larvae (*Chrysoperla carnea*), ladybird beetle (*Hippodamia convergens*), and a parasitic hymonoptera wasp (*Nasonia vitripennis*). These proteins showed no toxicity to these organisms when fed	Data on toxicity to other beneficial insects was not found in the sources consulted.	Data on toxicity to other beneficial insects was not found in the sources consulted.	Data on toxicity to other beneficial insects was not found in the sources consulted.

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	respectively. The LC_{50} for green lacewings exposed to Cry1F is ≤5.2µg/g diet which is 104x higher than the HEEE in nectar and >7x higher than that in pollen, and 4.68 µg Cry1Ac/g diet which is 94x higher than the HEEE in nectar and 2x higher than that in pollen. The LC_{50} for lady beetles exposed to Cry1F is >300 µg/g diet which is 428x higher than the HEEE in pollen, and to Cry1Ac is 4.68 µg/g diet which is 9x the HEEE in pollen. The LC_{50} for the parasitic Hymenopetra exposed to Cry1F is >5.2 µg/mL which is 104x higher than the HEEE in nectar and 7x higher than that in pollen, and to Cry1Ac is 46.8 µg/mL which is 900x higher than the HEEE in nectar and 19x higher than that in pollen. Monarch butterfly larvae were tested as a surrogate for indirect exposure of a hypothetical sensitive non-target lepidopteran larvae to cotton pollen. The EC_{50} for microbe-derived Cry1Ac protein is 0.9 ng/mL which is 10x higher than the EEC in pollen from Event 3006-210-23, and for microbe-derived Cry1F protein is 5,220 ng/mL which is >450,000x higher than the EEC in pollen from Event 281-24-236. Results from studies on	levels many times greater than the levels found in pollen and nectar of the cotton plants. Published nontarget insect abundance studies from observations in Cry1Ac cotton suggest no impact on the abundance of beneficial The LC_{50} of purified Cry1Ac Btk HD-73 crystals to the parasitic Hymenoptera *Nasonia* vitripennis, lady beetles and green lacewings was 10,000 and 1,700 times the amount found in nectar and pollen, respectively. insects. Parasitic Hymenotpera data was waived by the EPA due to lack of field exposure to Cry2Ab protein, and because of Cry2Ab2 specificity to Lepidoptera. The LC_{50} for purified Cry2Ab2 protein fed to green lacewing larvae is >1,100 ppm Cry2Ab2 protein and the LD_{50} is >4,500 ppm. The LC_{50} represents 5.5x the maximum concentration in corn plant material and 21.6x the maximum concentration in cotton plant material. Based on these results it can be concluded that green lacewing will not be adversely effected when exposed to Cry2Ab2 in the field. The LC_{50} for purified Cry2Ab2 protein fed to green lacewing larvae is >1,100 ppm Cry2Ab2 protein and the LD_{50} is >4,500 ppm. The LC_{50} 21.6x the maximum concentration in cotton plant material. Based on these results it can be			

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	beneficial arthropods present in field plots of MXB-13 cotton treated for nonlepidopteran pests showed no consistent adverse effects compared to nontransgenic cotton silimilarly treated or treated to control all insect pests.	concluded that green lacewing will not be adversely effected when exposed to Cry2Ab2 in the field.			
Soil-dwelling decomposer invertebrate toxicity	Soil organism exposure to Cry proteins from current transgenic crops may be via roots, soil incorporation of above ground plant parts after harvest, or by pollen deposited on the soil. Root exposure may occur by feeding or, theoretically, by ingestion or absorption after secretion of Cry proteins into the soil. Some soil components, e.g. clays and humic acids, bind Cry proteins in a manner that makes them less degradable by soil microorganisms, but without eliminating their insect toxicity. Therefore, exposure to soil-bound Cry protiens may also be an exposure route. Microbially-derived Cry1F and Cry1Ac protein, alone or in combination, showed no toxicity to earthworms. The LC_{50} was >247 mg for Cry1F which is 762x the EEC in soil and >107 mg Cry1Ac protein per kg (soil dry weight basis) which is 3,066x the EEC in soil. Laboratory studies on chronic effects of Cry1F and Cry1Ac alone or in combination on Collembola (*Folsomia candida*) showed no effect on survival	Cry1Ac and Cry2Ab were tested on collembola and Cry2Ab was tested on earthworms. No adverse effects were observed on these organisms at or above predicted concentrations in the soil to which they would be exposed. The LC_{50} for Cry1Ac against collembola is >200ppm. The 14-day LC_{50} for earthworms exposed to purified cotton Cry2Ab2 protein in soil was greater than 330 mg Cry2Ab2 mg protein/kg dry soil. The LC_{50} was determined to be >330 mg of Cry2Ab2 protein. No deleterious effect on earthworms is expected to result from the growing of Cry2Ab2 protein containing cotton plants. The LC_{50} to Collembola exposed to cotton leaf tissue in the diet was > 69.5 µg Cry2Ab2 protein/g diet. There were no adverse affects on the rate of Collembola reproduction. Cry2Ab does not pose a hazard to Collembola, a representative soil inhabiting species.	Data on toxicity to beneficial to soil organisms was not found in the sources consulted.	Data on toxicity to beneficial to soil organisms was not found in the sources consulted.	Data on toxicity to beneficial to soil organisms was not found in the sources consulted.

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	and reproduction. The EC_{50} for Cry1F was >702 mg which is 2,167x the EEC in soil and for Cry1Ac was >164.5 µg per kg diet which is 5x the EEC in soil.				
Mammalian toxicity	Because Cry1Ac and Cry1F proteins are contained in the plant, there is minimal potential for human exposure via dermal, eye, or inhalation exposure routes. In addition, human consumption of cotton products is limited. Typically, cotton-by-products occur as blended items and comprise a minor component of daily dietary intake. Proteins as a class are not highly toxic to humans, nor are they likely to bioaccumulate in fatty tissue or to persist in the environment. The Cry1 proteins used in crop production, either as formulated microbial sprays or as Plant Incorporated Protectants (PIPs), show no mammalian toxicity, do not correspond to known allergens, and are rapidly digestible. In a laboratory study, separate microbial protein preparations containing 14% Cry1Ac or 30% Cry1F were evaluated for acute oral toxicity to mice. All mice survived and there were no adverse effects during the two-week observation period. The LD_{50} in mice was >700 mg/kg for the Cry1Ac protein, and >600 mg/kg for the Cry1F protein.	The data submitted to EPA indicate no toxicity from Cry1Ac to rodents during the acute oral testing at the maximum hazard dose. These data showed a lack of toxicity to mammals from exposure to high levels of Cry1Ac. The LD_{50} of Cry1Ac HD-173 for mice is >4200 mg/kg body weight. The LD50 of Cry2Ab2 protein for mice is >1450 mg/kg body weight. The Cry2Ab2 protein is not stable to digest in simulated gastric fluid.	These pyrethroids are moderately toxic to mammals. **Cyfluthrin:** Considered moderately toxic to mammals via ingestion and inhalation. Chronic, reproductive, developmental, or teratogenic effects were unclear from the sources consulted. There was no evidence of carcinogenicity or mutagenicity. The LD_{50} ranged from > 100 mg/kg in dogs, 291 - 609 mg/kg in mice, >1000 mg/kg in sheep and rabbits. In inhalation toxicity tests with rats, the LC_{50} of cyfluthrin in air was >1,089 µg/L in 1 hour tests, and ranged from 469 - 592 µg/L in 4 hour tests. Although itis an irritant to human skin, it is not considered to have high dermal toxicity. The dermal for rats was > 5,000 mg/kg, and it is not a skin irritant or sensitizer in guinea pigs and rabbits **Lambda Cyhalothrin** is moderately toxic via the oral route in test animals. The reported rat LD50 for the technical product is 64 mg/kg. No data were available regarding the acute toxicity of the technical compound via the inhalation route, but the formulated product Karate is moderate to high toxicity via	Malathion is slightly to moderately toxic to mammals when ingested. Profenofos is moderately toxic. Methyl Parathion is highly toxic to mammals. **Malathion** is slightly toxic via the oral route. Reported oral LD_{50} values of 1000 mg/kg to >10,000 mg/kg in the rat, and 400 mg/kg to >4000 mg/kg in the mouse. It is also slightly toxic via the dermal route, with reported dermal LD50 values of greater than 4000 mg/kg in rats [2,13]. Effects of malathion are similar to those observed with other organophosphates, except that larger doses are required to produce them Several studies have documented developmental and reproductive effects due to high doses of malathion in test animals. However, malathion fed to rats at low dosages caused no reproductive effects. It is not likely that malathion will cause reproductive or teratogenic effects in humans under normal circumstances. Results from tests on mutagenicity are unclear. Available evidence suggests that malathion is not carcinogenic but the data are not conclusive. **Profenofos** results of acute and	Toxicity of **Spinosad** to mammals is very low. With respect to subchronic toxicity, spinosad was evaluated in 13-week dietary studies and showed NOEL's of 4.9 mg/kg/day in dogs, 6 mg/kg/day in mice, and 8.6 mg/kg/day in cats. No dermal or systemic toxicity occurred in a 21 day repeated dose dermal toxicity study in rabbits given the limit does of 1000 mg/kg/day. There was no evidence of carcinogenicity in two rodent species at all dosages tested. Mutagenicity studies showed no mutagenic activity associated with spinosad. There was no developmental effects observed in two oral developmental toxicity studies in rats and rabbits up to the highest dose tested (HDT).

	Cotton Events - Cry1A(c) (3006-210-23) Cry1F (281-24-236) and the Stacked product Cry1Ac/Cry1F (MXB-13)	Bollgard I® – Cry1Ac (Lines 531, 757, 1076) Bollgard II® – Cry2Ab (Event 15985)	Cyfluthrin (Baythroid) Lambda Cyhalothrin (Karate Z) Bifenthrin (Capture)	Malathion (Fyfanon) Profenofos (Curacron) Methyl Parathion	Spinosad (Tracer)
			the inhalation route, with a reported 4-hour inhalation LC50s of 0.175 - 0.315 mg/L for rats. The technical product has reported dermal LD50s of 632 - 696 mg/kg for rats. It is non-irritating to the skin of rabbits and non-sensitizing to the skin of guinea pigs but may cause mild eye irritation in rabbits. Karate, however, causes severe primary skin irritation in rabbits and mild skin sensitization in guinea pigs Lambda cyhalothrin is unlikely to cause chronic effects, nor reproductive, teratogenic, or mutagenic effects in humans under normal conditions, since no carcinogenic effects have been noted on various test animals. **Bifenthrin** is moderately toxic to mammals when ingested. It does not cause inflammation or irritation on human skin. The LD50 is about 54-70 mg/kg in rats. The LD_{50} for rabbits whose skin is exposed to bifenthrin is greater than 2,000 mg/kg. Bifenthrin does not sensitize the skin of guinea pigs. It is virtually non-irritating to rabbit eyes. No information was available on chronic toxicity. Reproductive, developmental, or teratogenic effects of bifenthrin were unclear from the sources consulted. There is inconclusive evidence of mutagenic effects. EPA has classified it as a class C carcinogen, a possible human	subacute oral, dermal, and inhalation studies on small mammals indicated that profenofos is moderately toxic. Eye contact studies with rabbits indicated moderate irritation and skin contact studies in rabbits indicated minimal irritation. No reproductive, developmental, or carcinogenic effects from profenofos were observed. **Methyl Parathion** is highly toxic via the oral route, with reported oral LD_{50} values of 6 to 50 mg/kg in rats. It is highly toxic via the dermal route as well. The 1-hour inhalation LC_{50} for methyl parathion in rats is 0.24 mg/L. Reproductive effects in humans are not likely under normal circumstances. Available evidence indicates that methyl parathion does not cause teratogenic, mutagenic, or carcinogenic effects.	

	Cotton Events - Cry1A(c) (3006-210-23) Cry1F (281-24-236) and the Stacked product Cry1Ac/Cry1F (MXB-13)	Bollgard I® – Cry1Ac (Lines 531, 757, 1076) Bollgard II® – Cry2Ab (Event 15985)	Cyfluthrin (Baythroid) Lambda Cyhalothrin (Karate Z) Bifenthrin (Capture)	Malathion (Fyfanon) Profenofos (Curacron) Methyl Parathion	Spinosad (Tracer)
			carcinogen.		
EPA Category		These Bt cottons are classified as Toxicity Category III pesticides and are labeled with the signal word CAUTION	These **Pyrethroid** insecticides must bear the signal word WARNING.	**Malathion** must bear the signal word CAUTION. **Profenofos** must bear the signal word WARNING. **Methyl parathion** is a highly toxic compound in EPA toxicity class I. Methyl parathion must bear the signal word DANGER.	**Spinosad** is classified as a Toxicity Category III pesticide and is labeled with the signal word CAUTION based on the acute dermal study.

References

Event 281-24-236, Cry1F: Cry1F Cotton Petition for Nonregulated Status, Mycogen/Dow petition 03-036-01p
Event 3006-210-23, Cry1Ac: Cry1Ac Cotton Petition for Nonregulated Status, Mycogen/Dow petition 03-036-01p

Bollgard I®, Cry1Ac: EPA Bt Plant-Pesticides Biopesticides Registration Action Document. Environmental Assessment, p. IIC1-IIC87.
Bollgard II®, Cry2Ab: Bt Plant-Pesticides Biopesticides Registration Action Document. Bacillus thuringiensis Cry2Ab2 protein and its genetic material necessary for its production in cotton.

Cyfluthrin: Extoxnet Extension Toxicology Network Pesticide Information Profiles (http://ace.orst.edu/info/extoxnet/pips)

Cyhalothrin: Extoxnet Extension Toxicology Network Pesticide Information Profiles (http://ace.orst.edu/info/extoxnet/pips)

Bifenthrin: Extoxnet Extension Toxicology Network Pesticide Information Profiles (http://ace.orst.edu/info/extoxnet/pips)

Malathion: Extoxnet Extension Toxicology Network Pesticide Information Profiles (http://ace.orst.edu/info/extoxnet/pips)

Profenofos: Syngenta Material Safety Data Sheet and label for Curacron 8E (http://www.syngentacropprotection-us.com); EPA Interim Reregistration Eligibility Decision (IRED) Document (http://www.epa.gov/oppsrrd1/REDs/2540ired.pdf)

Methyl Parathion: Extoxnet Extension Toxicology Network Pesticide Information Profiles (http://ace.orst.edu/info/extoxnet/pips)

Spinosad: EPA Pesticide Fact Sheet (http://www.epa.gov/opprd001/factsheets/spinosad.pdf)

Appendix D. Summary table of data submitted with the petitions in support of nonregulated status for Cotton Events Cry1F and Cry1Ac.

	Petition 03-036-01p for Cry1F Cotton Event 28-24-236	**Petition 03-036-02p for Cry1Ac Cotton Event 3006-210-23**
Molecular Genetic Characterization Data: Insertion, Inheritance and Expression of Transgenes		
Observed and predicted hybridizing fragments in Southern analysis of subject cotton event.	Table 4, pg. 23.	Table 4, pg. 23.
Southern blot of subject cotton events probed with *cry1F* or *cry1Ac* probe, respectively, indicates presence of single intact copy of the respective insecticidal transgene.	Fig. 3, pg. 25.	Fig. 3, pg. 24.
Southern blot of subject cotton events probed with mas or ubi probes demonstrates the presence of a single intact copy of the mannopine synthase promoter and the Ubi Zm1 promoter with their respective genes, *cry1F (synpro)* or *cry1Ac (synpro).*	Fig. 5, pg. 27.	Fig. 6, pg. 28.
Southern blot of subject cotton events probed with *pat* probe, indicates presence of single intact copy of the pat gene in both cotton events plus the presence of a second copy of pat in the Cry1F cotton event.	Fig. 4, pg. 26, and Mycogen/Dow Sept. 15 letter to APHIS, Fig. 17, pg. 3.	Fig. 4, pg. 25.
Southern blot of subject cotton events probed with *ubi* or *mas* probes demonstrates the presence of a two copies of the Ubi Zm1 promoter for the *pat* genes in Cotton Event Cry1F and one copy of the mannopine synthase promoter for the *pat* gene in Cotton Event Cry1Ac.	Fig. 6, Pg. 28 and Mycogen/Dow Sept. 15 letter to APHIS, Fig. 6, pg. 5.	Fig. 5, pg. 27.
Southern blot of subject cotton events probed with ORF25 probe indicates the presence of a single intact copy of the 3' bi-directional terminator for the *pat* and respective *cry1F (synpro)* or *cry1Ac (synpro)* genes.	Fig. 7, Pg. 29, and Mycogen/Dow Sept. 15 letter to APHIS, Fig. 7, pg. 7.	Fig. 7, pg. 29.
Southern blot analysis demonstrates stable inheritance of inserts of the *pat* genes and respective *cry1F (synpro)* or *cry1Ac (synpro)* genes across two generations of the subject cotton event.	Table 5, Pg. 30, and Fig. 8-9, pp. 31-32.	Table 5, Pg. 30, and Fig. 8-9, pp. 31-32.
Southern blot analysis demonstrates stable inheritance of inserts of the *pat* genes and respective *cry1F (synpro)* or *cry1Ac (synpro)* genes within a segregating generation of the subject cotton event, and that the *pat* gene and insecticidal genes co-segregate.	Fig. 10 and 11, pp. 34-35.	Fig. 10 and 11, pp. 34-35.

Molecular Genetic Characterization Data continued	**Petition 03-036-01p for Cry1F Cotton Event 28-24-236**	**Petition 03-036-02p for Cry1Ac Cotton Event 3006-210-23**
Southern blot of subject cotton events probed with *ery*^{R} probe demonstrates absence of the bacterial erythromycin resistance gene.	Fig. 12, pg. 37.	Fig. 12, pg. 37.
Chi Square analysis of the expressed Cry1F (synpro) or Cry1Ac (synpro), as detected by an immunoassay, across F1 and F2 and backcross generations demonstrates these genes segregate according to expected standard Mendelian genetics.	Table 7, and accompanying Fig.13, pp. 38-39.	Table 7, and accompanying Fig.13, pp. 38-39.
Summary of Cry1F (synpro), Cry1Ac (synpro) and PAT protein expression levels in various tissues of subject cotton events grown in several field sites.	Tables 8 and 9, pp. 41-42. See also and Mycogen/Dow Sept. 15 letter to APHIS for sample descriptions.	Tables 8 and 9, pp. 41-42. See also and Mycogen/Dow Sept. 15 letter to APHIS for sample descriptions.
Summary of Cry1F (synpro), Cry1Ac (synpro) and PAT protein expression levels in cotton seed and its processed fractions from the subject cotton events.	Table 10, pg. 43.	Table 10, pg. 43.
Environmental Characterization and Effects Data		
Field data reports from field trials conducted in the United States	Appendix 1	Appendix 1
Efficacy of subject cotton events (and stacked event) against target pests	Pg. 53 (Appendix 2, . Pellow J.W, 2002.)	Pg. 54. (Appendix 2, . Pellow J.W, 2002)
Agronomic characteristics of the subject cotton event and stacked product in comparison to the nontransgenic recurrent parent PSC355 used in backcrosses.	Table 13, pg. 44. See also the Mycogen/Dow Sept. 15 letter to APHIS for accompanying Table 13 A-C and additional calculations on seeds/boll and bolls/plant.	Table 14, pg. 55. See also the Mycogen/Dow Sept. 15 letter to APHIS for accompanying Table 14 A-C and additional calculations on seeds/boll and bolls/plant.
Summary of proximate analysis of cottonseed and processed fractions from subject cotton events compared to a nontransgenic control.	Table 14-15, pp. 55-56.	Table 15-16, pp. 56-57.
Studies indicating that Cry1F (synpro), Cry1Ac (synpro) and PAT do not exhibit characteristics commonly attributed to allergenic proteins.	Appendix 2, Stelman 2001, Korjagin 2001 and 2002, Herman and Gao, 2001.	Appendix 2, Stelman 2001, Korjagin 2001 and 2002, Herman and Gao, 2001.
Toxicant and anti-nutrient analysis of cottonseed, cottonseed oil, leaves and squares of the subject cotton event and nontransgenic control line.	Tables 16-19, pp. 57-60, and Appendix 2, Phillips et al., 2002.	Tables 17-20, pp. 58-61, and Appendix 2, Phillips et al.2002,.
Selectivity of microbially-derived Cry1F and Cry1Ac determined for insect species of different orders.	Pg. 62, and Appendix 2, Herman and Young, 1999, and Herman, 2001.	Pg. 63, and Appendix 2, Herman, 2001.

Environmental Characterization and Effects Data continued	**Petition 03-036-01p for Cry1F Cotton Event 28-24-236**	**Petition 03-036-02p for Cry1Ac Cotton Event 3006-210-23**
Estimated high end exposure estimates and environmental concentrations of insecticidal proteins from the subject cotton events and stacked product, and results of ecotoxicity studies on mammals, birds, soil invertebrates, aquatic organisms and nontarget arthropods of plant or microbially-derived Cry1F and /or Cry1Ac.	Pp. 62-69, Tables 22-23, , as well as accompanying studies in Appendix 2, and revised sections in Mycogen/Dow Sept. 15 letter to APHIS, including Table21,	Pp. 63-70, Tables 23-24, , as well as accompanying studies in Appendix 2, and revised sections in Mycogen/Dow Sept. 15 letter to APHIS, including Table22,
Characterization of the plant-expressed and microbially-derived Cry1F and Cry1Ac proteins used in the ecotoxicity studies, for amino acid sequence, molecular weight, peptide fragments, glycosylation, and bioequivalency against pests with different susceptibilities.	Fig. 14-16 and Tables 11-12 on pp. 43-50; Table 20 on pg. 61, and accompanying studies in Appendix 2	Fig. 14-18 and Tables 11-13 on pp. 43-50; Table 21 on pg. 62, and accompanying studies in Appendix 2
Field surveys to evaluate effects on non-target beneficial arthropods of Cry1F/Cry1Ac Bt cotton (the stacked product).	Pg. 71; Appendix 2, Mahill and Storer, 2002; and Storer, N.P. 2003, included in the Mycogen/Dow Nov.25 letter to APHIS.	Pg. 72; Appendix 2, Mahill and Storer, 2002; and Storer, N.P. 2003, included in the Mycogen/Dow Nov.25 letter to APHIS.
Overlay of endangered butterfly counties with cotton acreage.	Fig. 19	Fig. 20

Appendix E. Determination of Non-regulated Status for Cotton Transformation Events 281-24-236 and 3006-210-23

In response to petitions (designated 03-036-01p and 03-036-02p) from Mycogen Seeds c/o Dow AgroSciences LLC (Mycogen/Dow), APHIS has determined that genetically engineered cotton transformation events 281-24-236 and 3006-210-23, and progeny derived from these events, including those derived from crossing these events, will no longer be considered regulated articles under APHIS regulations at 7 CFR Part 340. (For the sake of simplicity, APHIS has referred to transformation events 281-24-236 and 3006-210-23 as Cotton Events Cry1F and Cry1Ac, respectively, in this Environmental Assessment. The OECD unique identifier for event 281-24-236 is DAS-24236-5 and for event 3006-210-23 is DAS-21023-5.) Permits or acknowledged notifications that were previously required for environmental release, importation or interstate movement under those regulations will no longer be required for these cotton transformation events or their progeny. Importation of seeds and other propagative material would still be subject to APHIS foreign quarantine notices at 7 CFR Part 319 and the Federal Seed Act regulations at 7 CFR Part 201.

This determination is based on APHIS' analysis of data and references provided in the petitions and other relevant information as described in this environmental assessment and in our response to the public comments submitted on these petitions and our environmental assessment. This analysis indicates that these transformation events, and progeny derived from them, will not pose a plant pest risk for the following reasons. (1) They exhibit no plant pathogenic properties – although a plant pathogen was used in the development of these cotton transformation events, the pathogen is no longer associated with these plants, and the plants do not contain genetic material from this pathogen that can cause plant disease. (2) They exhibit no characteristics that would cause them to be more weedy than their non-transgenic parent cotton line or other cultivated cotton. (3) Gene introgression from these transformation events to native, introduced, or naturalized species of *Gossypium* in the United States is unlikely to occur at a high frequency, and it is not likely to increase the weediness potential of any resulting progeny, nor adversely effect genetic diversity any more than would introgression from other cultivated cotton. (4) Disease and insect susceptibility of the transformation events were similar to the non-transgenic parental cultivar, with the exception of the expected increased resistance to targeted lepidopteran pests. Furthermore, the compositional profile of the seeds from these transformation events, or processed fractions derived therefrom, were not significantly different compared to their non-transgenic recurrent parent counterparts, and naturally occurring toxicants and anti-nutrients were found to be similar to the non-transgenic counterpart and/or within the acceptable ranges reported in the literature. Therefore, no direct or indirect plant pest effect on raw or processed plant commodities is expected. (5) Field observations, compositional analyses, estimates of exposure, and data on the safety of the engineered Bt Cry1F and Cry1Ac proteins and PAT proteins all indicate that these transformation events should not have a greater potential than other cultivated cotton to damage or harm organisms beneficial to agriculture. (6) Compared to current cotton pest and weed management practices, cultivation of these transformation events should not reduce the ability to control pests and weeds in cotton or other crops. In addition to our finding of no plant pest risk, there will be no affect on threatened or endangered species resulting from a determination of non-regulated status for these transformation events or their progeny.

APHIS also has concluded that there may be new varieties bred from transformation events 281-24-236 and 3006-210-23; however, they are unlikely to exhibit new plant pest properties, i.e., properties substantially different from any observed for cotton descended from these transformation events, or those observed for other cotton varieties not considered regulated articles under 7 CFR Part 340.

Mep C Hoffman for Cindy Smith

Cindy Smith
Deputy Administrator
Biotechnology Regulatory Services
Animal and Plant Health Inspection Service
U.S. Department of Agriculture
Date: JUL 15 2004