US Environmental Protection Agency
Office of Pesticide Programs

BIOPESTICIDES REGISTRATION ACTION DOCUMENT

Bacillus thuringiensis Cry2Ab2 protein and its genetic material necessary for its production in cotton)

□(Chemical PC Code 006487)□AMENDED

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

***Bt* Cry2Ab2 Bollgard II Cotton Registration Action Document**

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BACILLUS THURINGIENSIS CryAb2 Bollgard II Seed Increase REGISTRATION ACTION TEAM

Benefit Assessment

Edward Brandt, M.A.
Alan Reynolds, M.S.
Sharlene Matten, Ph.D.

Environmental Fate and Effects Risk Assessment

Zigfridas Vaituzis, Ph.D.
Chris Wozniak, Ph.D.
Robyn Rose, M.S.
Gail Tomimatsu, Ph.D.

Health Effects Risk Assessment

John Kough, Ph.D.
Michael T. Watson, Ph.D.

Insect Resistance Management

Sharlene Matten, Ph.D.

Registration Support

Willie Nelson, M.S.
Leonard Cole, M.S.

Biopesticides Registration Action Document Team Leader

Leonard Cole, M.S.

Office of General Counsel

Suzanne Krolikowski

Bt Cry2Ab2 Bollgard II Cotton Registration Action Document

I. Overview

A. Executive Summary

Monsanto has requested an amendment to change their seed increase registration of 20,000 acres to establish breeding nurseries and seed increase fields of Bollgard II event 15985 cotton to a full commercial section 3 registration. The seed increase acres were planted in Arizona (80 locations; 8,000 acres), Mississippi (32 locations; 11,700 acres) and Louisiana (3 locations; 300 acres). Health and ecological tests conducted in support of this seed increase registration as well as their amendment to change the seed increase registration to a full commercial section 3 registration are addressed in this review. This review applies to Monsanto's amendment to change their seed increase registration to a full commercial section 3 registration. The data submitted under the seed increase registration were also relied upon as part of the Agency's review for a full commercial registration. Additionally, required data such as a confirmatory heat stability study and insect resistance management data were reviewed and considered prior to EPA making its decision to amend this seed increase registration to permit full commercial use of the plant-incorporated protectant.

Monsanto Company first petitioned the Agency for an experimental use permit December 8, 1998. In April 2000, Monsanto petitioned the Agency for a full section 3 commercial registration for *Bacillus thuringiensis* delta endotoxin as produced by Cry2Ab2 and its controlling sequence as expressed in cotton. The Agency published in the Federal Register a notice of receipt of a new active ingredient (Cry2Ab2) in Bollgard II cotton on March 21, 2001 (Volume 66, Number 55; pp. 15867-15868). According to The Union of Concerned Scientists, comments pursuant to the publication of the notice of receipt were sent to the Agency; however, the Agency never received these comments. The Union of Concerned Scientists submitted via fax the original comments. These comments have been placed in the docket. Due to the nature of the comments, the Agency feels that there would be no adverse effects to human health or the environment. These comments will be addressed in detail in the FR Notice announcing the amendment to permit full commercial use of the pesticide. The comments requested that the Agency not grant Monsanto Company a registration for Bollgard II. In February 2002, Monsanto petitioned the Agency to amend the section 3 commercial registration to a seed increase/plant propagation registration. On June 14, 2002, the EPA granted Monsanto a conditional registration for seed increase/plant propagation for the plant-incorporated protectant Cry2Ab2 insect control protein in Bollgard II cotton. The Agency granted Monsanto Company an experimental use permit (EUP) for 800 acres in May of 1999 which expired in May 2000; subsequently, the Agency granted Monsanto an extension in May of 1999 which expired in May 2001. A third extension/expansion was granted in July 2001 for a maximum of 6200 Acres per year.

Bollgard II cotton expresses the *Bacillus thuringiensis* Cry2Ab2 protein stacked with already registered Cry1Ac protein. Bollgard II is intended to protect cotton from feeding by tobacco budworm (*Heliothis virescens*), pink bollworm (*Pectinophora gossypiella*), cotton bollworm (*Helicoverpa zea*), cabbage looper (*Trichoplusia ni*), saltmarsh caterpillar (*Estigmene acrea*),

cotton leaf perforator (*Bucculatrix thurbeiella*), soybean looper (*Pseudoplusia includens*), beet armyworm (*Spodoptera exigua*), fall armyworm (*Spodoptera frugiperda*), yellowstriped armyworm (*Spodoptera ornithogolli*) and European corn borer (*Ostrinia nubilalis).*

B. Use Profile

- Active Ingredient: *Bacillus thuringiensis kurstaki* Delta-Endotoxin as Produced by the Cry2Ab2 Gene and Its Controlling Sequences as Expressed in Cotton.
- **Trade and Other Names: BollGard II®**
- **OPP Chemical Code: 006445**
- **Basic Manufacturer: Monsanto Company**
  700 Chesterfield Parkway North
  St. Louis, MO 63017
- **Type of Pesticide: Plant-incorporated protectant**
- **Uses: Cotton**
- **Target Pest(s): Cotton bollworm, tobacco budworm, pink bollworm saltmarsh caterpillar, cotton leaf perforator, soybean looper, beet armyworm, fall armyworm, yellowstriped armyworm and European corn borer (*Ostrinia nubilalis).***

C. Regulatory History

Monsanto Company has developed Bollgard II cotton plants which contain two proteins, i.e., stacked proteins consisting of Cry1Ac and Cry2Ab2 proteins. These insect control proteins are derived from the common soil microbe *Bacillus thuringiensis* subsp. *kurstaki*. The new biotech cotton plants were developed using particle acceleration plant transformation procedures to insert the Cry2Ab2 insect control gene into an existing Bollgard cotton variety expressing the Cry1Ac protein. Monsanto Company petitioned the Agency to amend the seed increase registration to a full commercial section 3 registration on May 16, 2002; subsequently, the company also requested that the EPA extend its seed increase registration for an additional period of time to allow for the development and submission of the outstanding data requirements.

In the Federal Register of October 10, 1997, (62 FR 52998) (FRL-5748-5), EPA issued a notice pursuant to section 408 of the Federal Food, Drug, and Cosmetic Act, (FFDCA), 21 U.S.C. 346a(d), as amended by the Food Quality Protection Act (FQPA) (Public Law 104-170) announcing the filing of pesticide tolerance petition, petition number 7F4888, by Monsanto

Company. This notice included a summary of the petition prepared by the petitioner Monsanto Company. There were no comments received in the response to the notice of filing. The petition was for exemption from the requirement of a temporary tolerance when used as plant-incorporated protectants (PIPs) in the food and feed commodities of field corn, popcorn, cotton seed, cotton oil, cotton meal, cotton hay, cotton hulls, cotton forage, and cotton gin by products. The temporary tolerance exemption was issued by EPA on April 27, 2001, and is due to expire May 1, 2004. A permanent tolerance exemption has also been issued for residues of Cry1Ac when used as a plant-incorporated protectant in or on all raw agricultural commodities (60 FR 47489, Sept 13, 1995).

II. Science Assessment

A. Product Characterization

The characterization data submitted by the registrant provides adequate product information to guide the risk assessment. This section provides information specifically on the Cry2Ab2 protein including isolation, purification and confirmation of the Cry2Ab2 protein expressed in bacteria is similar to that found in cotton. Further, this section addresses stability of the protein and levels of *Bt* expression for various plant tissues. Specific information and data are included in descriptive and tabular formats.

Several methods were used to confirm that the identity of the Cry2Ab2 protein produced by fermentation and used in the toxicity testing was the same as that produced in the cotton plant. The EC_{50} and LC_{50} with a pest insect (*Helicoverpa zea*), protein purity, and protein stability were determined. The protein as tested appeared to have the expected molecular weight (a 63 kDa protein band) by gel electrophoresis, was relatively pure, immunoreactive with appropriate antibodies, and stable through the 87 day time point. The tested substance was lyophilized Cry2Ab2 protein powder (Lot# 6312829) isolated from *Bacillus thuringiensis* strain EG7699.

A solution of Cry2Ab2 protein (approximately 1, 2, and 3 : g total protein) was applied to a polyacrylamide gel (4->20%) run under reducing conditions. Molecular weight markers were used to determine the weight of the Cry2Ab2 and contaminant proteins. Densitometric analysis was used to determine the relative percent of Cry2Ab2 protein and contaminant proteins. Protein molecular weight was estimated by comparison to marker proteins. The purity of the ~63 kDa protein (Cry2Ab2) was estimated to be 65.5% of total protein.

Immunoblots were prepared and developed separately with either polyclonal anti-Cry2Ab2 rabbit antibody or monoclonal anti-Cry2Aa mouse antibody. One major protein (~63kDa) was recognized by both polyclonal anti-Cry2Ab2 antibody and monoclonal antibodies raised against Cry2Aa. An additional immunoreactive protein (~53 kDa) likely represents a degradation product of the 63 kDa protein.

The N-terminus of the major polypeptide in Cry2Ab2 was determined to coincide to a large extent with the predicted sequence. The registrant indicates that a “ragged N-terminus” resulted

in the identification of a major and minor sequence. This result may have been caused by "protease-sensitive"sites at the N-terminus of the protein. Further, the registrant indicates that the cysteine at position 13 was not observed in either determined sequence, consistent with the Edman degradation chemistry used in this method in which cysteine residues are chemically unstable.

The stability of Cry2Ab2 in purified water was determined at storage temperatures of 4, -20 and -80° C over a period of 87 days. Aliquots were removed at 0, 11, 41, 52, and 87 days and analyzed using SDS-PAGE. Based upon these gels, the protein is stable for at least 87 days stored at -80, -20 and 4 °C in purified water. Densitometric analysis was also performed on the SDS-PAGE gels. Only the samples stored at 4 °C showed even a small decrease in OD, the samples stored at -80 and -20 °C did not show significant degradation.

The results of the product characterization testing are summarized in Table 1 below.

Table 1. Summary of Cry2Ab2 Protein Properties

Criteria	Method	Result
Identity and Molecular Weight	a) N-terminal sequence analysis b) Immunoblot	a) confirmed b) confirmed
Concentration	Protein assay and amino acids compositional analysis	correction factor of 1.7 was established
Strength	CEW bioassay (corrected for purity and amino acid compositional analysis)	EC_{50} of 0.24 : g/ml LC_{50} of 52.4 : g/ml
Purity	Densitometry	65.5%
Stability	SDS-PAGE and immunoblot analysis of solutions stored at 4, -20 and -80° C	≥ 87 days at -20 and -80° C; at least 52 days at 4° C
Heat Stability	SDS-PAGE/Western Blot analysis of samples	no bands seen after treatment at $121 C for 30 mins

The data provided for Cry2Ab2 indicate that the tested protein was relatively pure (65.5%) and readily recognized by protein-specific antibodies (Cry2Ab2-specific polyclonal antibodies). Further confirmation of the protein was accomplished by N-terminal sequencing. A Cry2Aa monclonal antibody was also used for detection and analysis as it cross-reacts with Cry2Ab2. Although no specific information was provided regarding the specificity of this antibody, confirmation via Cry2Ab2 polyclonal antibody and N-terminal sequence further confirm the Western blot results using the monoclonal antibody. Overall, the protein identity confirmation using several methods confirm the identity of the protein

Expression of Cry2Ab2 in cotton tissues using ELISA: The level of Cry2Ab2 protein in cotton tissue is important to determine both for human exposure and nontarget organisms assessments and for insect resistance management. The levels were determined by use of ELISA on extracted cotton tissue samples. Cry2Ab2 specific rabbit polyclonal antibody was immobilized onto 96-well microtiter plates. The ELISA was a one-step procedure which included simultaneous incubation of the sample with rabbit anti-Cry2Ab2 polyclonal antibody conjugated to horseradish peroxidase (HRP). Each plate was developed with TMB (3,3',5,5'-tetramethylbenzidine) and the concentration of Cry2Ab2 protein was determined by extrapolation of absorbance against a serially diluted 7-point standard curve with values between 1.56 and 100 ng/ml.

Protein Samples in Cotton Plant Samples: The Cry2Ab2 protein tissue expression results are summarized below. The mean level of each protein as well as the range is shown for each cotton line. Data for the 28 through 108 days post-planting were provided for the leaf tissue only. The following units are microgram/gram fresh weight (ug/gfwt).

Tissue	Cotton Line	Mean	28 Days Post Planting	55 Days Post Planting	85 Days Post Planting	108 Days Post Planting
Leaf	15813	11.3±5.3 (4.55-16.3)	17.3±4.41 (11.6-20.9)	28.1±2.98 (26.0-32.5)	9.87±2.30 (6.50-11.62)	9.14±1.30 (7.86-10.74)
	15985	23.8±6.3 (10.1-33.3)	21.0±4.9 (15.5-24.9)	40.1±(6.5) (34.6-49.4)	19.7±2.7 (15.9-21.8)	16.7±0.6 (15.8-17.3)
Seed	15813	37.1±5.5 (24.7-45.6)				
	15985	43.2±5.7 (31.8-50.7)				
Pollen	15813	1.17±0.07 (1.12-1.22)				
	15985	<0.25				
Whole Plant	15813	4.15±1.00 (2.45-5.50)				
	15985	8.80±1.20 (7.28-10.46)				

The protein levels contained in the table above show that the detection and quantitation techniques employed are adequate for Cry2Ab2 protein (as well as other proteins described).

B. Human Health Assessment

The health effects assessment concludes that there is a reasonable certainty that no harm will result from exposure to Cry2Ab2. The human health assessment for the Cry2Ab2 draws heavily on the science and toxicology of proteins. Cry2Ab2 is a insecticidal protein found in spore crystals of *Bacillus thuringiensis* subsp. *kurstaki* (*Btk*). *Btk* and its crystal proteins (including both Cry1Ac in strain HD-73 and Cry2Ab2 in strain HD-1) have been approved for use in food as microbial pesticides and have been present in products that have been used without significant adverse human health effects. These same proteins when expressed in plants are expected to behave similar to normal dietary proteins. A *B. thuringiensis* strain expressing more than one type of crystal protein could be expected to have synergistic or additive effects on the intended target pest insect. However, there is no indication from the testing of microbial *B. thuringiensis* strains registered and known to express an array of crystal proteins that human dietary safety has been adversely changed. While the human dietary safety is expected to remain unchanged by the addition of Cry2Ab2 to cotton expressing Cry1Ac, EPA notes it is also unlikely that there is any significant dietary exposure to any cotton protein in cottonseed oil, the only cotton derived component directly consumed by humans.

1. Mammalian Toxicity and Allergenicity

a. Mammalian Toxicity

Mammalian toxicology data are available to examine the potential effects of Cry2ab2 protein on human health and assess if the data support registration of *Bacillus thuringiensis* Cry2Ab2 delta-endotoxin and the genetic material necessary for its production in cotton. *Bt* microbial pesticides, containing Cry proteins other than Cry2Ab2, have been applied for more than 30 years to food and feed crops consumed by the U.S. population. These data would also support other Cry2Ab2 plant-incorporated protectants' human health assessments provided adequate information was submitted to show that the Cry2Ab2 test material derived from microbial cultures were biochemically and functionally similar to the proteins produced as the plant-incorporated protectant ingredients.

Monsanto submitted information which adequately described the Cry2Ab2 delta-endotoxin from *Bt*, as expressed in cotton, along with the genetic material necessary for its production. Because it would be difficult, or impossible, to extract sufficient biologically-active toxin from the plants to perform toxicology tests, Monsanto used delta-endotoxin produced in bacteria. Product analysis data was submitted to show that the microbially expressed and purified Cry2Ab2 delta-endotoxin is sufficiently similar to that expressed in the plant to be used for mammalian toxicological purposes. Plant and microbially produced Cry2Ab2 delta-endotoxin were shown by these studies to have similar molecular weights and immunoreactivity (SDS-PAGE and Western blots), to lack detectable post-translational modication (glycosylation tests), to have identical amino acid sequences in the N-terminal region and to have similar results in bioassays against Heliothis virescens and Helicoverpa zea. While it is difficult to prove that two proteins are identical, the combined results of the above studies indicate a high probability that these two sources produce proteins that are essentially identical by available protein analytical assays.

Acute Oral Toxicity

The acute oral toxicity data demonstrates that the Cry2Ab2 endotoxin is non-toxic to humans**.** There do not appear to be adverse effects resulting from oral exposure to the Cry2Ab2 protein at 1450, 359 and 67 mg/kg bodyweight. There were two unscheduled deaths, in animals that were part of the control groups, apparently due to punctured esophagi at dosing. In addition, many gross findings were described from the necropsy, but these findings occurred at equal frequency in both the control and test groups and do not appear to result from exposure to the Cry2Ab2 protein. No other apparent adverse affects were identified through the course of the study and therefore the protein appears to cause no significant adverse effects via oral exposure at a level of at least 1450 mg/kg bodyweight, the highest does tested.

Guideline No	Study	Results	MRID
885.3050	Acute Oral Toxicity Study	This study shows that there appears to be no adverse effects in mice resulting from oral exposure of Cry2Ab2 protein. Further, no other adverse effects were identified through the course of study and the protein appears to be safe via oral exposure at a level of at least 1450 mg/kg bodyweight. The study is acceptable and assigned Category III.	449666-02
	In vitro Digestibility	This study shows that Cry2Ab2 protein is not stable to digest in simulated gastric fluid. This study is acceptable.	449666-03

b. Allergernicity

Digestibility of Cry2Ab2 protein

Incubation of Cry2Ab2 protein in simulated gastric fluid (SGF) (pH 1.2) resulted in the loss of detectable protein prior to the 15 second observation point indicating that Cry2Ab2 is not stable to digestion *in vitro* as determined by SDS-PAGE/Western blot and insect bioassay. Incubation of the protein in simulated intestinal fluid resulted in a 50 kDa protein digestion product.

Protein stability in simulated digestive fluids, specifically SGF, is one characteristic of food allergenic proteins which have been described. The tests performed show that Cry2Ab2 protein is not stable to digestion in simulated gastric fluid. Incubation of Cry2Ab2 in SGF results in the loss of detectable protein by the 15 second observation point as detected by SDS-PAGE/Western blot and insect bioassay. Incubation in the SIF resulted in a 50 kDa digestion product that was stable throughout the 24 hour observation point. Using SDS Western Blot Analysis of samples, the heat stability study confirmed that there is no potential allergenicity to this protein at temperatures greater than or equal to 120 degrees centigrade.

Amino Acid Homology

Several amino acid database comparison tools were employed to compare the amino acid sequence of Cry2Ab2 to known protein allergens and gliadins (simple proteins found in seeds that are insoluble in absolute alcohol or water). The database was compiled to allow for comparison of Cry2Ab2 to these proteins. The protein with the greatest similarity was only 32.7% identical and 57.1% similar over a 49 amino acid stretch. This level of similarity does not indicate significant similarity to any of the proteins or gliadins contained in the database. In addition, no contiguous stretch of 8 identical amino acids was identified in either the FASTA or IDENTITYSEARCH algorithms suggesting a lack of immunological similarity. Based upon these data, it does not appear that Cry2Ab2 shares significant structural, biological or immunological similarity with known protein allergens or gliadins.

Other amino acid database comparison tools were employed to compare the amino acid sequence of Cry2Ab2 to known protein toxins. The TOXIN4 database was compiled to allow for comparison of Cry2Ab2 to these proteins. All of the protein similarities identified were to insecticidal protein, with no similarity to proteins known to be toxic to humans and/or animals. Based upon these data, there is no evidence that Cry2Ab2 shares significant structural, biological or immunological similarity with known protein toxins, other than those affecting insects.

c. Mutagenicity and Developmental Toxicity, Subchronic Toxicity, and Chronic Exposure and Oncogenicity Assessment

The lack of mammalian toxicity at high levels of exposure demonstrates the safety of the product at levels above possible maximum exposure levels. This is similar to the Agency position regarding toxicity and the requirement of residue data for the microbial *Bacillus thuringiensis* products from which this plant-incorporated protectant was derived. [See 40 CFR Sec. 158.740(b).] For microbial products, further toxicity testing to verify the observed effects and clarify the source of the effects (Tiers II & III) and residue data are only triggered by significant acute effects in studies such as the mouse oral toxicity study. Tiers II & III were not triggered because there is no potential for oncogenic effects.

The acute oral toxicity data submitted support the determination that the Cry2Ab2 protein is non-toxic to humans. When proteins are toxic, they are known to act via acute mechanisms and at very low dose levels (Sjoblad, *et al*., 1992). Since no effects were shown to be caused by the plant-incorporated protectants, even at relatively high dose levels, the Cry2Ab2 delta-endotoxin protein is not considered toxic. Because these proteins break down into their constituent amino acids almost immediately upon ingestion, there would be no chronic exposure to the protein and therefore no need for chronic toxicity testing. Because there is no chronic exposure, the mutagencity, developmental toxicity, subchronic toxicity, chronic exposure and oncogenicity assessment studies are not required.

2. Effects on the Immune System

There were no observed effects of Cry2Ab2 on the immune system seen in the acute oral toxicity study and no indication from the structural analyses cited above to indicate that Cry2Ab2 protein shares any of the biochemical features common to known food allergens.

3. Effects on the Endocrine System

The pesticidal active ingredients are proteins, derived from sources that are not known to exert an influence on the endocrine system. Therefore, the Agency is not requiring information on the endocrine effects of these plant-incorporated protectants.

4. Dose Response Assessment

No toxicological endpoints are identified so no dose response assessment can be done.

5. Dietary Exposure and Risk Characterization

a. Toxicity and Allergenicity Conclusions

The data submitted and cited regarding potential health effects for the Cry2Ab2 protein include information on the characterization of the expressed Cry2Ab2 delta-endotoxin in cotton, the acute oral toxicity, and the *in vitro* digestibility and heat stability of the delta-endotoxin. The results of these studies were determined to be adequate to evaluate human risk and the validity, completeness, and reliability of the available data from the studies were considered.

b. Acute and Chronic Dietary Risks For Sensitive Subpopulations Particularly Infants and Children

FFDCA section 408(b)(2)(C) provides that EPA shall assess the available information about consumption patterns among infants and children, special susceptibility of infants and children to pesticide chemical residues and the cumulative effects on infants and children of the residues and other substances with a common mechanism of toxicity. In addition, FFDCA section 408 provides that EPA shall apply an additional tenfold margin of exposure (safety) for infants and children in the case of threshold effects to account for pre- and post-natal toxicity and the completeness of the database unless EPA determines that a different margin of exposure (safety) will be safe for infants and children.

In this instance, based on all the available information, the Agency concludes that infants and children will consume minimal residues of this plant-incorporated protectant and, for those residues that they do consume, that there is a finding of no risk because of lack of toxicity. The use sites for Cry2Ab2 delta endotoxins are all agricultural for control of lepidopteran insects. Therefore, exposure via residential or lawn use to infants and children is not expected. Even if negligible exposure should occur, the Agency concludes that such exposure would present no risk due to the lack of toxicity.

Thus, there are no threshold effects of concern and, as a result the provision requiring an additional margin of safety is not required. Further, information on consumption patterns, special susceptibility, and cumulative effects are not useful here.

c. Aggregate Exposure (Not Including Occupational Exposure) Risk Conclusions

The Agency has considered available information on the aggregate exposure levels of consumers (and major identifiable subgroups of consumers) to the pesticide residue and to other related substances. These considerations include dietary exposure under the tolerance exemption and all other tolerances or exemptions in effect for the plant-incorporated protectants residue, and exposure from non-occupational sources. Exposure via the skin or inhalation is not likely since the plant-incorporated protectants are contained within plant cells which essentially eliminates these exposure routes or reduces these exposure routes to negligible. Oral exposure, at very low levels, may occur from ingestion of processed products and drinking water. However, a lack of mammalian toxicity and the digestibility of the plant-incorporated protectants has been demonstrated.

d. Cumulative Effects Risk Conclusions

The Agency has considered available information on the cumulative effects of such residues and other substances that have a common mechanism of toxicity. These considerations included the cumulative effects on infants and children of such residues and other substances with a common mechanism of toxicity. Because there is no indication of mammalian toxicity to these plant-incorporated protectants, there are no cumulative effects.

e. Occupational, Residential, School and Day Care Exposure and Risk Characterization

Exposure via the skin or inhalation when the cotton is growing is not likely since the plant-incorporated protectants are contained within plant cells which essentially eliminates these exposure routes or reduces these exposure routes to negligible. Worker exposure to the Cry protein via seed dust is also expected to be negligible because of the low amount of protein expressed in transformed plants. If such exposure should occur, the Agency concludes that such exposure would not be expected to present any risk due to the lack of toxicity. However, if any unreasonable adverse effects caused by exposure to Cry2Ab2 is identified, these effects must be reported to the Agency as described in Sec. 6(a)(2) of FIFRA.

f. Aggregate Exposure from Multiple Routes Including Dermal, Oral, and Inhalation

The Agency has considered available information on the aggregate exposure levels of consumers (and major identifiable subgroups of consumers) to the pesticide residue and to other related substances. These considerations include dietary exposure under the tolerance exemption and all other tolerances or exemptions in effect for the plant-incorporated protectants residue, and exposure from nonoccupational sources. Exposure via the skin or inhalation is not likely since the plant-incorporated protectants are contained within plant cells which essentially eliminates these exposure routes or reduces these exposure routes to negligible. At very low levels, oral exposure may occur from ingestion of processed cotton products and drinking water and inhalation exposure may occur to workers exposed to cotton seed. However, a lack of mammalian toxicity and the digestibility of the plant-incorporated protectants has been

demonstrated. All use sites for Cry2Ab2 delta endotoxin are agricultural. Therefore, exposure via residential or lawn use to infants and children is not expected. Even if negligible exposure should occur, the Agency concludes that such exposure would present no risk due to the lack of toxicity.

6. Food Clearances/Tolerance Exemptions for Stacked Product

All active and inert ingredients resulting from the use of Bollgard II are currently covered by the following tolerance exemptions:

Bacillus thuringiensis Cry2Ab2 protein and the genetic material necessary for its production in corn or cotton are exempt from the requirement of a tolerance when used as a plant-pesticide in the food and feed commodities of corn, sweet corn, popcorn, cotton seed, cotton oil, cotton meal, cotton hay, cotton hulls, cotton forage and cotton gin byproducts.[40 CFR 180.1215; 66 FR 24066, May 1, 2001]

Bacillus thuringiensis subspecies *kurstaki* CryIA(c) delta-endotoxin and the genetic material necessary for its production in are exempt from the requirement of a tolerance when used as a plant-pesticide in all plant raw agricultural commodities.[40 CFR 180.1155; 62 FR 17722, April 11, 1997]

There is a reasonable certainty that no harm will result from aggregate exposure to the U.S. population, including infants and children, to the Cry 2Ab2 protein and the genetic material necessary for its production. This includes all anticipated dietary exposures and all other exposures for which there is reliable information. The Agency has arrived at this conclusion because no toxicity to mammals has been observed for the plant-incorporated protectants and anticipated exposures are negligible.

f. Combined effects of Cry1Ac and Cry2Ab2 proteins

EPA has made a resonable certainty of no harm finding for both Cry1Ac and Cry2Ab2 proteins. EPA has granted tolerance exemptions to cover both proteins and their residues. EPA believes that human dietary safety is not expected to change as a result of exposure to a product containing both these proteins (Cry1Ac and Cry2Ab2). See previous discussion (part II. B).

C. ENVIRONMENTAL ASSESSMENT

1. Ecological Effects Hazard Assessment

The ecological assessment section of this document focuses heavily on evaluating the impacts of Cry2AB2 plant-incorporated protectants on non-target species. Specific data are cited for concerns related to fate in soils and potential indirect effects on soil biota, direct effects on non-target species including, avian and aquatic species, insects, and endangered or threatened species. The results are presented in both descriptive and tabular format.

The Agency has determined that the non-target organisms most likely to be exposed to the protein in transgenic cotton fields were beneficial insects feeding on cotton pollen and nectar, upland birds feeding on cotton seed and soil invertebrates. Thus, toxicity tests were required utilizing representatives of those organisms. The toxicity of the Cry2Ab2 protein has been evaluated following challenge of several species of vertebrates and invertebrates, including: northern bobwhite quail, catfish, adult and larval honeybees, a parasitic hymenopteran (*Nasonia*), green lacewings, Ladybeetle beetles, earthworms, and collembola. Waterfowl, freshwater and estuarine/marine fish, and aquatic invertebrate tests (*Daphnia*) were waived due to lack of substantive exposure. Since Cry2Ab2 is an insect toxin that has never shown any toxicity and/or pathogenicity to plant species, terrestrial and aquatic plant studies have also been waived.

Wild mammal hazard assessment is being performed on the basis of rodent toxicity data prepared for human health risk assessment purposes. The data submitted to the Agency indicate no toxicity to rodents during the acute oral testing at the maximum hazard dose. These data show a lack of toxicity to mammals from exposure to high levels of Cry2Ab protein. Therefore no further wild mammal testing is required.

Summary of Non-Target Organism Toxicity Testing of Cry2Ab2

Guideline No	Study	Results	MRID NO
885.4050	Avian Testing	Although the NOEC for Cry2Ab2 in ground cottonseed was 100,000 ppm, such a low % cottonseed meal in the diet is not representative of all poultry diets. Adequate data to make an assessement for time limited registration was presented. A six week study with appropriate proportions of cottonseed meal in the diet is required to assess hazards to domesticated fowl from continuous exposure to higher levels of Cry2Ab2 protein.	450863-16
885.4200	Freshwater Fish Testing	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.	450863-18 453371-03
850.6200	Earthworm Testing	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 no observed effect concentration was determined to be greater than 330 mg of Cry2Ab2 protein. No deleterious effect on earthworms is expected to result from the growing of Cry2Ab2 protein containing cotton plants.	450863-13
885.4380	Honey Bee Adult and Larval Testing	(NOEC) for purified cotton Cry2Ab2 protein fed to honey bee larvae (*Apis mellifera*) is >100 : g/mL (ppm). There were no adverse effects on larval survival to capping, adult emergence, and adult honey bee survival.	453371-02 450863-07 450863-08
885.4340	Parasitic Hymenoptera Larva	The study showed unacceptable control mortalities. A repeat study was waived due to lack of field exposure to Cry2Ab	450863-10

	Testing	protein, and because of Cry2Ab2 specificity to Lepidoptera.	
885.4340	A Dietary Toxicity Study with Green Lacewing Larvae	The NOEC for purified Cry2Ab2 protein fed to green lacewing larvae is >1,100 ppm Cry2Ab2 protein and the LD_{50} is >4,500 ppm. The NOEC 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.	450863-09
885.4340	A Dietary Toxicity Study with the Lady Beetle	The LC_{50} for adult lady beetles feeding on Cry2Ab2 protein is >4,500 ppm which is a significantly greater level than would be encountered in the field. However, a NOEC to lady beetle larvae cannot be determined from this study. Lady beetle larvae would potentially have a higher exposure to Cry2Ab2 than adults. Therefore a dietary toxicity study should be conducted to determine the NOEC for lady beetle larvae.	450863-11
885.4340	Chronic Collembola Toxicity Study	The NOEC to Collembola exposed to cotton leaf tissue in the diet was > 69.5 : g 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.	450863-14

a. Avian Testing

This study was conducted in accordance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency, Office of Pesticide Programs in 40 CFR Part 160 with certain exceptions that did not affect the integrity of the test. This study was conducted based on OPPTS Series 885.4050 Nontarget Avian Testing, Tier I. The study is scientifically sound and no treatment mortality or behavior change was observed between the dosed and control replicates.

The dietary LC_{50} for Cry2Ab2 protein in cottonseed meal when fed to juvenile northern bobwhite for 5 days was determined to be greater than 100,000 ppm diet because no toxicity was observed at this level. Because 100,000 ppm was the highest dose tested, EPA has determined that no observed effect concentration (NOEC) is also greater than 100,000 ppm. These data show that there will be no adverse effects on avian wildlife from incidental field exposure to Cry2Ab2 protein. These data, however, are not sufficient to make a hazard assessment from repeated avian exposure to higher doses of Cry2Ab2 in their diet. A 10% cottonseed meal in the diet is not representative of all poultry diets. A six week study with appropriate proportions of cottonseed meal in the diet is required to assess hazards to domesticated fowl from continuous exposure to higher levels of Cry2Ab2 protein. Therefore this study is classified as supplemental.

b. Freshwater Fish Testing

This study was conducted in accordance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency, Office of Pesticide Programs in 40 CFR Part 160 with certain exceptions that did not affect the integrity of the test. This is a non-guideline study based on Nontarget Freshwater Fish Testing (OPPTS Series 885.4200), Tier I.

In an 8 week feeding study, no toxicity was observed in channel catfish consuming a diet containing 20% cottonseed meal from Bollgard II with the Cry2Ab2 protein. Because 20% cottonseed meal containing Cry2Ab2 protein was the highest dose tested, EPA has determined that the dietary LC_{50} and the NOEC for Cry2Ab2 protein in cottonseed meal when fed to channel catfish for 8 weeks is greater than 20% of the diet. The data indicate that cottonseed meal derived from genetically modified cotton lines, 15813 and 15985 (Cry2Ab2) can be used as a feed ingredient in channel catfish diets up to levels of about 20% without adverse effects on fish growth, feed conversion efficiency, survival, behavior, or body composition. The lack of adverse effects may be due in part to the significant reduction in the concentration of the Cry2Ab2 protein in the modified cottonseed as compared to raw cottonseed prior to commercial processing of cottonseed (toasting). However, a similar study performed with corn meal which contained Cry2Ab2 protein that was not denatured (MRID 450863-19) showed no adverse effects on catfish at 20%.

d. Nontarget Invertebrate - Earthworm Testing

The study was conducted in compliance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency, Office of Pesticide Programs in 40 CFR Parts 160 and 792; Organization for Economic Development (OECD) Principles of Good Laboratory Practice; and Japan Ministries of Agricultural Forestry and Fisheries (MAFF), with certain exceptions that did not affect the integrity of the test. The testing was conducted based on OPPTS Series 850.6200 Earthworm Subchronic Toxicity Test and OECD Guideline 207.

The 14-day LC_{50} for earthworms exposed to Cry2Ab2 protein in an artificial soil substrate was determined to be greater than 330 mg Cry2Ab2 mg protein/kg dry soil; the no observed effect concentration was determined > 330 mg Cry2Ab2 mg protein/kg dry soil, the highest concentration tested. The study was procedurally sound and the data show that no adverse effects to earthworms are expected at Cry2Ab2 levels 12 and 83 times higher than the maximum expected environmental concentration for corn and for cotton respectively. Thus, an observable deleterious effect on earthworms is not expected to result from the growing of Cry2Ab protein containing cotton plants. This study meets current testing requirements for assessing subchronic risks to earthworms from plant-incorporated protectants derived from *Bacillus thuringiensis*.

e. Non-Target Arthropod Invertebrate Testing:

1. Honey Bee Larvae

This study was conducted in accordance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency, Office of Pesticide Programs in 40 CFR Part 160 with certain exceptions that did not affect the integrity of the test. An acceptable study was conducted based on OPPTS Series 885-4380, Honey bee testing Tier I.

It can be determined from this study that the no-observed-effect concentration (NOEC) for Cry2Ab2 protein fed to honey bee larvae (*Apis mellifera*) is >100 : g/mL (ppm) (MRID 453371-02). The test was scored for survival to capping, adult emergence, and adult survival. The larvae developed into adult honey bees normal in behavior and appearance. A NOEC could not be

determined from the results of an additional study submitted for review (MRID 450863-07). However, results from this study supplement results from MRID 453371-02 in demonstrating a lack of risk from larval honey bees feeding on Cry2Ab2 protein.

2. Adult Honey Bee Testing

This study was conducted in accordance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency, Office of Pesticide Programs in 40 CFR Part 160 with certain exceptions that did not affect the integrity of the test. This study was conducted based on OPPTS Series 885-4380, Honey bee testing Tier I. This study showed the no-observed-effect concentration (NOEC) for Cry2Ab2 protein fed to adult honey bees (*Apis mellifera*) is >68 : g/mL Cry2Ab2 protein.

Cry2Ab2 protein showed no measurable deleterious effects on honey bee larvae and adults up to the level tested.

3. Parasitic Hymenoptera Larva Testing

This study was conducted in accordance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency, Office of Pesticide Programs in 40 CFR Part 160 with certain exceptions that did not affect the integrity of the test. This study was conducted based on OPPTS Series 885-4340 Nontarget Insect Testing, Tier I.

The guidelines recommend terminating the test when 20% mortality is reached in the control group or after 30 days. Since this study was terminated prematurely, an additional study should be conducted that continues for 30 days or until 20% mortality is reached in the assay control group. However, there was a high rate of morality in the assay control group; equal to the mortality in the 100 ppm potassium arsenate reference group which suggests that there was a non-treatment related effect occurring. This test should have been conducted until 20% mortality was achieved in the vehicle control group or for 30 days as described in OPPTS 885.4340. Due to the high rate of mortality in the assay control and 220 ppm Cry2Ab2 protein treatment group, and premature termination of the study, an LC_{50} could not be determined.

On April 18, 2002, Monsanto submitted a letter to the Agency requesting a waiver from parasitic Hymenoptera toxicity testing. This waiver request was based on a lack of exposure of parasitic Hymenoptera to the Cry2Ab2 protein. In addition, parasitic Hymenoptera are not expected to be susceptible to Cry2Ab2 since it is highly specific against lepidopterans and dipterans. Due to the lack of exposure and susceptibility of parasitic Hymenoptera to the Cry2Ab2 protein expressed in cotton or corn, the Agency has accepted Monsanto's request to waive this data requirement.

4. Green Lacewing Larva Testing

This study was conducted in accordance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency, Office of Pesticide Programs in 40 CFR Part 160 with certain exceptions that did not affect the integrity of the test.

This study was conducted based on OPPTS Series 885-4340 Nontarget Insect Testing, Tier I except the test was terminated when 50% pupation was reached in the assay control group. The guidelines recommend terminating the test when 20% mortality is reached in the control group or after 30 days. However, it is known that younger larvae are more susceptible to *Bt* proteins than older larvae. It can be assumed that adverse effects related to green lacewing larvae feeding on Cry2Ab2 protein would be observed once 50% pupation occurred. Based on this study, the no-observed-effect concentration (NOEC) for Cry2Ab2 protein fed to green lacewing larvae is >1,100 ppm Cry2Ab2 protein and the LD_{50} is >4,500 ppm. The NOEC 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.

5. Ladybeetle Beetle Testing

This study was conducted in accordance with Good Laboratory Standards as published by the U.S. Environmental Protection Agency, Office of Pesticide Programs in 40 CFR Part 160 with certain exceptions that did not affect the integrity of the test. This study was conducted based on OPPTS Series 885-4340 Nontarget Insect Testing, Tier I.

The primary route of exposure to Cry2Ab2 protein by Ladybeetle beetle adults and larvae would be from cotton pollen ingestion. Since some of beetles in the treatment and control groups were observed to be immobile/and or lethargic, a NOEC cannot be determined from this study. However, it can be concluded that the LC_{50} for adult Ladybeetle beetles feeding on Cry2Ab2 protein is >4,500 ppm which is a significantly greater level than would be encountered in the field.

This study does not adequately show that there will not be a hazard to Ladybeetle beetle populations from Cry2Ab2 because lethargic/immobile effects were observed. In addition, Ladybeetle beetle larvae would potentially have a higher risk of exposure to Cry2Ab2 than adults. Therefore, a dietary toxicity study should be conducted to determine the NOEC for Ladybeetle beetle larvae.

6. Collembola feeding on Cotton Tissue

Although this study was not conducted in accordance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency, Office of Pesticide Programs in 40 CFR Part 160, The Agency has determined that the study is scientifically valid. This study was conducted based on OPPTS Series 885-4340 Nontarget Insect Testing, Tier I.

This study determined that the presence of Cry2Ab2 protein was not toxic to Collembola. Cry2Ab2 protein also did not adversely affect the rate of Collembola reproduction. Mortality demonstrated in the positive control group and observations of green digestive tracts in the other groups verified that Collembola are ingesting the test cotton tissue material. Results of this study showed the no-observed-effect concentration (NOEC) of Collembola exposed to Cry2Ab2 protein from cotton leaf tissue in the diet was > 69.5 : g Cry2Ab2 protein/g diet. This study adequately addresses potential concerns for Cry2Ab protein expressed in transgenic cotton to Collembola (*Folsomia candida*) a representative of beneficial soil insect species. The results of

this study demonstrate that Cry2Ab proteins found in transgenic cotton pose no hazard to soil inhabiting Collembola species, and by inference to other beneficial soil insects.

f. Combined effects of Cry1Ac and Cry2Ab proteins.

Bollgard II contains both Cry1Ac and Cry2Ab2 proteins. Nontarget testing with Cry1Ac (006445) and Cry2Ab2 proteins separately did not show any hazard to nontarget species. Any unexpected synergistic effects from Bollgard II which produces both Cry1Ac and Cry2Ab2 proteins are not anticipated because no adverse effects were seen in several nontarget tests (avian, earthworm and collembolla species) which were performed on tissue containing both Cry proteins.

g. Endangered Species Considerations

Based on the submitted Cry1Ac and Cry2Ab2 protein toxicity and exposure data there will not be a "may effect" situation for endangered or threatened mammals, birds, plants and aquatic species to these plant-incoporated protectants. The nontarget testing confirms the expectation that Cry1Ac and Cry2Ab2 protein toxicity is confined to Lepidoptera species larvae; therefore, non-lepidopteran endangered or threatened species will not be affected by these proteins. Cotton is insect pollinated and pollen containing the Cry protein is not likely to drift out of fields. Nevertheless, relatively high Cry1Ac and Cry2Ab2 dosages were not toxic to the test species representative of organisms likely to be exposed to such pollen (e.g. Ladybeetle beetles, green lacewings, honeybees). In addition, the larvae of endangered Lepidoptera species in cotton growing counties (Quino Checkerspot butterfly, Riverside County CA; Saint Francis' Satyr butterfly, Cumberland and Hoke Counties, NC and Kern Primrose Sphinx moth, Kern County CA) are not going to be exposed to the Cry proteins because their habitats do not overlap with cotton fields (e.g. the Quino Checkerspot butterfly is found only in the coastal sage scrub habitat in southern California, the Kern Primrose Sphinx moth is found only on a privately owned ranch in Walker Basin, Kern County, California, and the only known populations of Saint Francis' Satyr butterfly are found in wetlands dominated by sages and grasses on Government property in North Carolina) and their larvae do not feed on cotton and will not be exposed to Cry protein in pollen. The amount of pollen that would drift from these cotton plants onto plants fed upon by endangered/threatened species, would be very small compared to the levels fed to the test species. Therefore, EPA does not expect a “may effect” scenario to any endangered/threatened species and therefore, if any exposure, the levels would be so low as to not effect the lepidopterans from cotton containing the Cry1Ac and Cry2Ab2 protein.

h. Nontarget Effects Summary

Considering all of the information available, the weight of evidence indicates no unreasonable adverse effects of Cry1Ac and Cry2Ab singularly or jointly expressed in cotton to non-target wildlife, plants, beneficial invertebrates, or listed endangered/threatened species from the proposed seed increase registration. Two follow-up studies will be required as a condition of amended seed increase commercial registration.

i. Ecological Effects Data Gaps

1. Avian Testing. In October of 2000 the SAP recommended that, for a final risk assessment, studies that strengthen the hazard analysis are needed. EPA agreed with this recommendation and in its *Bt* Crop Reassessment of October 2001 required that avian subchronic studies be generated by the *Bt* corn registrants. EPA believes a 10% cottonseed meal in the diet is not representative of all poultry feeds. Thus, EPA is imposing a requirement to repeat this avian study with higher levels of the Cry2Ab2 protein. A six week study with appropriate proportions of cottonseed meal in the diet is requisite to assess hazards to domesticated fowl from continuous exposure to higher levels of Cry2Ab2 protein. Because EPA does not believe that this data requirement was reasonably foreseeable by the applicant, the Agency has decided to grant an amended seed increase commercial registration while such study is being conducted.

2. Ladybeetle Beetle Testing. The submitted study does not adequately show that there will not be a hazard to lady beetle populations from Cry2Ab2 because lethargic/immobile effects were observed. However, because the LC_{50} for adult beetles feeding on Cry2Ab2 protein exceeds 4,500 ppm and this greatly exceeds the level in the field, EPA concludes there is not an unreasonable adverse effect to lady beetle adults. EPA also believes, however, that lady beetle larvae would potentially have a higher risk of exposure to Cry2Ab2 than adults. Therefore, a dietary toxicity study will be required to determine the NOEC for lady beetle larvae. As EPA has not previously required such a lady beetle *larvae* study for other registered PIP products, EPA does not believe that the applicant would reasonably have foreseen this data requirement. For this reason, EPA has decided to grant an amended seed increase commercial registration while such study is being conducted.

D. ENVIRONMENTAL FATE

1. Gene Flow

REFER TO THE ORIGINAL BOLLGARD II BRAD AND REFERENCES THEREIN

2. Fate of Cry2Ab2 Protein in Soil

Soil organisms may be exposed to *-endotoxins from current transgenic crops by exposure to roots, incorporation of above ground plant tissues into soil after harvest, or by pollen deposited on the soil. Root exposure may occur by feeding on living or dead roots or, theoretically, by ingestion or absorption after secretion of *-endotoxin into the soil. In addition, evidence suggests that some soil components, e.g. clays and humic acids, bind *-endotoxins in a manner that makes them recalcitrant to degradation by soil microorganisms, but without eliminating their insect toxicity. Therefore, exposure to *-endotoxin bound to soil particles may also be a route of exposure for some soil organisms.

A Cry protein DT_{50} (time to 50% degradation) study was submitted for registration of Bollgard II cotton containing Cry2Ab2 and Cry1Ac (MRID 453371-01). According to this study,

Cry2Ab2 + Cry1Ac proteins degrade rapidly in this sandy loam soil (typical soil type for cotton production). The DT_{50} was 2.3 days, DT_{90} was 15 days, and 75% of the protein degrades in the first week of incubation. However, this study uses the cotton bollworm (*Helicoverpa zea*) as the indicator species in the insect bioassay. The cotton bollworm is 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. An accurate degradation time (DT_{50}) cannot be determined from this study since there is not a high dose of Cry2Ab2 or Cry1Ac expressed to control the cotton bollworm.

A soil degradation study should be conducted for an unlimited full Section 3 registration with a species that is highly sensitive to the Cry2Ab2 protein (e.g., tobacco budworm; *Heliothis virescens*). Several studies indicate that Cry proteins bind to clays and humic acids, thus, slowing the rate of microbial degradation of these toxins compared to when these soil components are not present. Soil degradation studies should be conducted with soils high in clay and humic acid in addition to sandy loam soils since soils high in clay and humic acid represents a worst case scenario. In addition, the presence of the Cry1Ac protein should be considered in the data analysis. The Agency does not believe that the soil degradation study is needed at this time because of limitations on the time and acreage due to the destruction of the seed production. Consequently, there are no forseeable adverse effects to the environment. This registration will expire on May 1, 2004 at which time the time limited tolerance expires.

E. Insect Resistance Management

Insect resistance management strategies need to account for both Cry1Ac and Cry2Ab being pyramided in Bollgard™ II cotton lines.

1. Pest Biology

Knowledge of pest biology is critical for the development of effective IRM strategies. For example, refuges must be designed with a solid understanding of the target pest to maximize the production of susceptible insects and increase the likelihood of random mating between susceptible and potentially resistant pests.

TBW, CBW, and PBW differ in their impact on cotton on a regionally-specific basis. For example, in the Southeast, CBW is the predominant pest. In the Midsouth (Mississippi Delta), TBW is the most important pest; whereas, PBW is the only lepidopteran pest of importance in Arizona and California. However, there are many parts of the cotton belt in which TBW and CBW are both significant economic pests.

Key literature information (Caprio and Benedict 1996) regarding pest biology, adult movement, mating behavior, gene flow, and alternate hosts for TBW, CBW, and PBW has been reviewed previously by the Agency in its 1998 White Paper on *Bt* plant-pesticide resistance management (US EPA 1998) and most recently, in its 2001 Bt Plant-Incorporated Protectants Biopesticides Registration Action Document (USEPA 2001).

Based on the published research, TBW and CBW are highly mobile insects, with CBW being more mobile than TBW. Both TBW and CBW are polyphagous, but the utilization and effectiveness of alternate hosts has not been sufficient to prove that non-cotton hosts are effective refuges. PBW has limited mobility and dispersal (although it has extensive spring flights) and limited host range. Additional information is needed to further address larval and adult movement, mating behavior and dispersal, ovipositional preferences, population dynamics, gene flow, survival and fecundity, fitness costs, and the use of alternate cultivated or wild hosts as refuges. The varied cropping systems for cotton, including local and regional differences, should also be considered for evaluating the biology, ecology, and population dynamics and genetics of the target pests. This research will improve the strength and reliability of an IRM plan to effectively reduce the likelihood that TBW, CBW, or PBW will become resistant to the Cry1Ac delta-endotoxin. Therefore, for Bollgard cotton, the Agency made the determination that some additional IRM data are needed to characterize better the impact of alternate hosts and supplemental insecticide treatments on refuge effectiveness, and north-south movement of CBW (USEPA 2001, see Section III. "Bt Cotton Confirmatory Data and Terms and Conditions of the Amendment" and Registration Decision Memorandum dated September 29, 2001). These same data requirements should also apply to Bollgard II cotton.

2. Insecticidal Activity and High Dose Determination

Insecticidal Activity Against Lepidopteran pests

Monsanto has provided the results of *in vitro* and *in planta* studies of the efficacy of the Cry1Ac and Cry2Ab proteins. Both proteins are highly active against the three primary target lepidopteran pests of cotton: TBW, CBW, and PBW. The level of insecticidal activity against certain pests for either Cry1Ac and Cry2Ab is summarized in Table 1 below. There are some differences in insecticidal activity of these proteins against the secondary lepidopteran pests such as fall armyworm (FAW), beet armyworm (BAW), and soybean looper (SL). Cry2Ab has some greater activity against FAW and BAW than Cry1Ac, but Cry1Ac is more efficacious against TBW and CBW (see Table 1 below).

Table 1. Relative insecticidal toxicity activity of Cry1Ac and Cry2Ab
(modified from Table 1, p. 15, MRID# 455457-01)

Family/Species	**Cry1Ac (LC_{50} in ppm)**	**Cry2Ab (LC_{50} in ppm)**
PBW	0.006	0.1
CBW	1.56	15.26
TBW	0.035	0.62
FAW	>100	47.5
BAW	>100	19.4

Family/Species	**Cry1Ac (LC_{50} in ppm)**	**Cry2Ab (LC_{50} in ppm)**
SL	0.725	0.752

Bollgard II cotton, which expresses both the Cry1Ac and the Cry2Ab proteins, exhibits substantially higher control of all target species than does Bollgard cotton, which expresses Cry1Ac alone. The data provided in Appendix 4 (MRID# 455457-01) indicate that the insecticidal activity of the combination of proteins is increased over either protein tested alone. These data also demonstrate that both the Cry1Ac and Cry2Ab proteins are present at consistently high levels across all plant parts for the duration of the growing season. This means that the insect pests feeding on Bollgard II cotton would be exposed to both of the insecticidal proteins simultaneously.

Monsanto (MRID# 450293-01, January 28, 2000 submission) has analyzed data involving the influence of Bollgard cotton on secondary lepidopteran pests: cabbage looper (*Trichoplusia ni* Hubner), soybean looper (*Pseudoplusia includens* Walker), saltmarsh caterpillar (*Estigmene acrea* Drury), cotton leafperforator (*Buccalatrix thurberiella* Busk), and European corn borer (*Ostrinia nubilalis* Hubner). Based on the analysis of Cotton Insect Loss Surveys from 1996 through 2000, no change in the secondary status of these pests was observed nationally or regionally. Further study of how Bollgard and Bollgard II cotton and insect resistance management plans have impacted or will impact secondary lepidopteran pests is recommended.

Bollgard II High Dose Determination for TBW, CBW, and PBW

Monsanto has provided laboratory studies to demonstrate that the Cry2Ab protein alone and the Cry2Ab + Cry1Ac proteins as expressed in Bollgard II produce a functional "high dose" in Bollgard II cotton for control of CBW, TBW, and PBW. These studies will be discussed below. EPA has previously concluded that a moderate, non-high dose of Cry1Ac is produced in current Bollgard lines to control CBW and a functional high dose of Cry1Ac is produced to control TBW and PBW (USEPA 1998, 2001).

The level of Cry2Ab expression measured in the ELISA is greater than 10 times the level of Cry1Ac expression seen in Bollgard II plants (mean levels were 3.5-fold greater) (see MRID# 455457-01, Appendix 4, Figure 6). This relationship is seen for all sites, sampling times, and tissue types. The expression of Cry2Ab in Bollgard II plants does not appear to compromise the expression of Cry1Ac levels. That is, the level of expression of Cry1Ac in Bollgard II cotton is essentially the same as in Bollgard cotton. Higher overall expression of Cry2Ab2 compensates for its lower unit activity against the target pests. Overall, the data suggest that the co-expression of the two insecticidal proteins, Cry2Ab and Cry1Ac, is likely to result in increased and prolonged lepidopteran activity in all tissue types, especially in the reproductive tissues.

TBW

Insecticidal activity against TBW was measured in Bollgard II cotton tissues in field trials conducted in 1998 and 1999 to assess the efficacy of Bollgard II cotton against the TBW as compared to the efficacy of Bollgard cotton using a quantiative bioassay (i.e., measured in Cry1Ac equivalents per protein-specific ELISA assays described in Greenplate 1999). The mean insecticidal activity was generally 3.5 times higher, but at least 2.5 times higher, than for Bollgard cotton in all plant tissues (see MRID# 455457-01, Appendix 4, Figures 1-5). These increased insecticidal activity levels can be seen at all sites, sampling times and in all tissue types. Lower insecticidal activity in Bollgard II tissues was observed in large leaves compared to terminal or square activity, but this activity was still higher than in any Bollgard tissue.

EPA (USEPA 1998, 2001) and two SAPs (1998, 2001) have previously concluded that the Cry1Ac in Bollgard cotton represents a high dose against TBW**.** Data presented by Monsanto show that the Cry2Ab protein in Bollgard II carries even more insecticidal activity than the Cry1Ac protein in Bollgard II cotton. Therefore, Cry2Ab in Bollgard II represents a high dose against TBW. Thus, Bollgard II cotton expresses a high dose of Both Cry1Ac and Cry2Ab proteins against TBW.

PBW

The relative PBW activity of Cry1Ac (LC_{50} = 0.006) is greater than Cry2Ab (LC_{50} = 0.1). PBW is more sensitive to the Cry1Ac and Cry2Ab proteins than TBW (see Table 1 above). EPA (USEPA 1998, 2001) and two SAPs (1998, 2001) have previously concluded that the Cry1Ac in Bollgard cotton represents a high dose against PBW**.** Data presented by Monsanto show that the Cry2Ab protein in Bollgard II carries even more insecticidal activity than the Cry1Ac protein in Bollgard II cotton. Since there is a high dose for both of these proteins for TBW, it logically follows that there is also a high dose of these same proteins for PBW. Thus, Bollgard II cotton expresses a high dose of both Cry1Ac and Cry2Ab proteins against PBW. Data by Marchosky et al. (2001) collected from field trials, conducted in 2000 to assess efficacy and yield, indicate that the Bollgard II cotton lines achieved a level of control about one order of magnitude higher than the Bollgard comparison lines (at least 99% control). In addition, data for cotton lines expressing just the Cry2Ab protein showed these lines to be as least as effective against PBW as Bollgard cotton lines containing only the Cry1Ac protein.

CBW

EPA (USEPA 1998, 2001) and two SAPs (1998, 2001) have previously concluded that the Cry1Ac in Bollgard cotton (expressing only Cry1Ac) represents only a moderate (non-high) dose against CBW. Monsanto presents three separate sets of laboratory studies to demonstrate that the Cry2Ab protein alone and the Cry2Ab + Cry1Ac proteins are expressed at a "high dose" in Bollgard II cotton for control of CBW. These three methods taken together provide a strong case that the Cry2Ab protein represents a high dose against CBW. (Sharlene Matten, Ph.D., October 24, 2002). For more information please refer to Dr. Matten's review entitled "EPA Review of Monsanto Company's Bollgard II Cotton Insect Resistance Management Plan For Section 3 Full Commercial Registration [Reg. No. 524-522; Submissions: S607615 and

Sequence Homology of Cry1A Versus Cry2A Proteins

Based on information presented by Monsanto, Cry1A and Cry2A proteins share less than 20% sequence homology. Crickmore et al. (1998) indicate that the Cry1A and Cry2A classes are among the most divergent. Tabashnik et al. (1996) show that Cry2Aa2 clusters in a group distant from Cry1A toxins in a domain II loop on an amino sequence similarity dendogram examining cross-resistance potential of the diamondback moth. Previous work examining insect resistance to Bt indicate that when cross-resistance occurs, it occurs when the proteins are structurally similar and the insecticidal mechanisms are also similar (reviewed in Ferré and Van Rie, 2002). When proteins are dissimilar, as are Cry1A and Cry2A, it is likely that the insecticidal mechanisms would be different. Research by Jurat-Fuentes and Adang (2001) on domain II supports this conclusion. That is, toxins with low homology to Cry1A toxins in domain II loops are reasonable alternative toxins to Cry1A toxins in Bt crops or in Bt microbial formulations. Thus, lack of sequence homology supports the hypothesis that there will be a low likelihood of cross-resistance in the target insect pests for the Cry1Ac and Cry2Ab proteins.

Structural Comparison of Cry1Ac and Cry2Ab Proteins

Monsanto provides arguments that support the conclusion that the low likelihood of substantial sequence similarity between the Cry1Ac and Cry2Ab proteins suggests that there is a difference in their tertiary structure. There were two compelling pieces of information presented. Morse et al (2001) determined the three-dimensional crystal structure of the Cry2Aa toxin and defined the putative receptor binding epitope on the toxin. Their work indicates that the three-dimensional structure of Cry2A proteins are very different from Cry1A proteins. Cry2Ab (one of the toxins of interest in Bollgard II) shares 87% sequence identity with Cry2Aa (Widner and Whiteley, 1989). A second piece of evidence is provided by Kolwyck et al (2000). Their research showed that anti-Cry2Ab antibodies do not cross-react with the Cry1Ac proteins, nor do the anti-Cry1Ac antibodies cross-react with the Cry2Ab2 protein. Lack of cross-reactivity shows that the epitope binding sites for antibody recognition are different and therefore the tertiary structure is different. Lack of similar tertiary structure supports the conclusion that there will be a very low likelihood of high levels of cross-resistance in the target insect pests for the Cry1Ac and Cry2Ab proteins.

Mechanism of Action and Binding Characteristics

Cross-resistance is most likely when toxins share key structural features, which allows one resistance mechanism to confer resistance to more than one toxin. This is, if two separate Bt toxins bind to the same midgut receptor or share more than receptor , the likelihood of cross-resistance increases. In their submissions, Monsanto provides information from the literature that support the finding that Cry1Ac and Cry2A proteins do not have the same mechanism of action and binding characteristics. While some low level of cross-resistance is possible, it is

unlikely that high levels of cross-resistance would be conferred by resistance to Cry1A or Cry2A toxins because of the difference in their binding characteristics and mechanism of action.

English et al. (1994) concluded that binding characteristics of cotton bollworm to Cry1A and Cry2A toxins were different. These authors demonstrated that Cry2Aa did not bind to a specific, high affinity receptor that was capable of binding of Cry1Ac. Binding of Cry2Aa was non-saturable regardless of the amount of toxin added. Monsanto also included unpublished work by English (Monsanto letter, August 16, 2002) that examined the binding of Cry2Ab and Cry1Ac proteins to target insect gut brush border membrane vesicles (BBMV) in CBW, TBW, and PBW using the BIACORE 2000 instrument (Piscataway, NJ) and a hydrophobic sensor chip (L1). The BBMV were pretreated with 1% bovine serum albumin (BSA) prior to each assay to block non-specific protein binding. No specific binding was observed between the full-length Cry2Ab protein and any BBMV of CBW, TBW, and PBW. This research indicates that Cry2Ab, like Cry2Aa, does not exhibit specific binding kinetics in the presence of BBMV. This additional work supports the conclusion that the Cry2Ab protein, and Cry2 proteins in general, produce highly potent ion channels to compensate for binding either to themselves or to a large collection of non-specific binding sites. Proteolytic digestion experiments using BBMV isolated from CBW and TBW showed that the Cry2Ab protein does not have a trypsin- or chymotrypsin-resistance core as described for the Cry1Ac protein and other Cry1 proteins. Conversely, proteolytic treatment of the Cry1Ac protein resulted in removal of the insecticidally inactive carboxyl terminal half of the protein and a small amino terminal region to yield a stable core protein of approximately 60 kDa. Proteolysis (using trypsin) has a positive impact on the ability of the Cry2Ab protein to form ion channels. Collectively, these studies demonstrate that the Cry1Ac and Cry2A proteins differ significantly with respect to presence of a protoxin, saturable binding kinetics and pore formation.

Activity of Cry2Ab Against Cry1A-resistant Colonies

Monsanto provided a series of studies examining the activity of Cry2Ab against Cry1A-resistant colonies. This evidence indicates that when Cry1A-resistant colonies are challenged with Cry2Ab that the potential for cross-resistance is low in TBW (Appendix 1), in CBW (Appendix 2), and in PBW (Appendix 3). Based on the information presented below, there is a low likelihood of cross-resistance (especially for high levels) in the target insect pests for the Cry2Ab and Cry1Ac proteins.

Gould (Appendix 1 of MRID# 455457-01) examined the adaptation of highly-resistant or broadly-resistant TBW colonies to the Cry1Ac toxin to Cry2Ab alone or to Cry2Ab + Cry1Ac. These studies showed no survivorship of the YHD2 (>20,000-fold resistant to the Cry1Ac toxin) on cotton tissue expressing Cry2Ab or both the Cry2Ab and Cry1Ac proteins. A second colony (KCB) had lower resistance to Cry1Ac and resistance was relatively broad-based. When these insects were place on plant tissue expressing both the Cry1Ac and Cry2Ab proteins, few or no insects survived. The few survivors did not develop beyond the first instar.

Bradley et al. (Appendix 2 of MRID# 455457-01) used one laboratory-selected CBW colony selected on Cry1Ac (13 generations) to examine potential cross-resistance. Their data indicate

that for the lab-selected resistant strain, 47% survived on conventional cotton compared to 19% on Bollgard cotton. However, when the lab-selected resistant strain was tested against the Bollgard II cotton lines, less than 5% of the larvae survived. No fruit penetration was observed in Bollgard II cotton by the lab-selected resistant strain.

Work with TBW and CBW resistant (to Cry1Ac) colonies indicates that there is some low potential for cross-resistance and that there are likely to be a range of Bt resistance mechanisms. Previously, published research indicates that there is evidence for broad cross-resistance (low levels of resistance) to Cry1A and Cry2A in laboratory-selected strains of beet armyworm (Moar et al. 1995) and TBW (Gould et al. 1992). Preliminary bioassays conducted on PBW by Dennehy et al. (Appendix 3 of MRID# 455457-01) showed that resistance to Cry1Ac in a resistant PBW strain (AZP-R) does not appear to confer cross-resistance to Cry2Ab. There were no survivors of the AZP-R strain on Bollgard II cotton tissue (Event 15985, the leading event to be commercialized).

3. Resistance Management Models for Pyramided Traits

Resistance simulation models predict that the greatest benefits of combining toxins in single plants by "pyramiding" or "stacking" are achieved when no cross-resistance occurs, when there are no fitness costs, when resistance to each toxin is rare and recessive, and when a refuge of plants without toxins are present. Modeling simulations of two-gene products predict that the resistance risk associated with a two-gene product will be significantly less than for a single-gene product (for example, Caprio 1998; Roush 1998; Hurley 2000). Monsanto concludes that modeling simulations predict that the two-gene product will have a life expectancy greater than six-fold compared to a single-gene product. This, they indicate, will add a degree of conservatism to the currently required IRM program for Bollgard.

Pyramiding relies on the idea that each protein is used individually in a way that would kill all insects susceptible to that protein, and in so doing, kills insects that are resistant to the companion protein (Roush, 1998). This has been described as "redundant killing" in the sense that most of the population is susceptible to both proteins and thus is killed twice. The extent to which the individuals that are resistant to one protein are killed by the other is central to the effectiveness of the pyramiding strategy.

Given that there are two insecticidal proteins, Cry1Ac and Cry2Ab, which have different modes of action, there is a very low likelihood of cross-resistance to Cry1Ac and Cry2Ab. Most likely, there would have to be multiple mechanisms of Bt resistance that occur in the field for Bollgard II to fail. If there is no cross-resistance, then the use of proteins jointly in a pyramided variety (assuming 70% mortality of RS heterozygotes for each protein) is considerably better in delaying resistance than the use of each protein sequentially (i.e., introduction of one protein after another) (see Roush 1998, Figure 2). These simulations indicate that a two-protein pyramid with a 5% structured (unsprayed) refuge can delay resistance for as long as if the two proteins are deployed sequentially with a 30% structured (unsprayed) refuge. That is, there is a six-fold advantage observed for the two-protein pyramid versus the single-protein sequential introductions. Thus, this conservative model illustrates the advantage of two-gene products

over single-gene products as long as the control of susceptible insects is high. Based on the high dose determinations above, Bollgard II produces a high dose of Cry2Ab for control of TBW, CBW, and PBW, a high dose of Cry1Ac for control of TBW and PBW, and a moderate dose of Cry1Ac for control of CBW. This means that the control of susceptible TBW and PBW by Cry2Ab and Cry1Ac is very high; while, the control of susceptible CBW by Cry2Ab is very high and by Cry1Ac is more moderate. Even without a high dose for CBW in the case of Cry1Ac, when both the Cry2Ab and the Cry1Ac are pyramided together, Bollgard II should still have the predicted advantages of the pyramid for delaying resistance because it is expected that at least 50% of the heterozygotes will be killed (see discussion in Roush 1998). Thus, pyramiding two or more proteins into a cultivar increases the chance that at least one of the proteins will be especially favorable to resistance management. Modeling simulations predict that pyramids (with high mortality) can reduce the need for larger refuges (Roush 1998, Hurley 2000, Livingston et al. 2002). A reduction in refuge size, under the ideal conditions of the pyramid (no other single-gene products) offers growers an easier opportunity for grower compliance (Hurley 2000 and Livingston et al. 2002). A pyramid may also reduce the reliance by cotton growers on maize and other hosts as refuge for *Helicoverpa* species (Roush 1998).

The durability of the pyramid is dependent on when the pyramided varieties are released (see Roush 1998, Figure 4). If the initial resistance allele frequencies are still low, a greater advantage can be gained for early introduction of the pyramided varieties. For Bollgard II cotton, this means that the initial resistance allele frequencies for Cry1Ac and Cry2Ab would have to be low to maximize the greatest advantage. Bollgard cotton varieties expressing the Cry1Ac protein have been commercialized since the 1996 growing season (seven years). Research by Burd et al. (2000) in North Carolina indicated that CBW resistance to the Cry1Ac protein may be inherited as a single dominant or partially dominant trait and that the resistance allele frequency has been estimated to be 4.3×10^{-4} (Burd et al. 2001). Burd et al. (2001) also estimated the resistance allele frequency for Cry2Ab to be 3.9×10^{-4}. Modeling simulations using these resistance allele frequencies indicate greater than a 3-fold advantage for the pyramid (e.g., Cry2Ab + Cry1Ac) over the single-protein products (Cry1Ac alone (Bollgard) or Cry2Ab alone (Bollgard II segregant)), i.e, 65 generations v. 20 generations (see Roush 1998, Figure 4,).

How quickly the resistance management benefits of a two-gene product are realized will depend upon the speed of introduction. It is expected that some overlap among Bollgard cotton (one gene = Cry1Ac), Bollgard II cotton (two genes = Cry2Ab and Cry1Ac) and potentially, other transgenic Bt cotton varieties will occur in the next five or more years. Livingston et al. (2002, unpublished) used a stochastic, spatial model of population and genetic dynamics to simulate resistance evolution in CBW to both Bt corn and Bt cotton varieties that express one or two proteins in eastern North Carolina, a mixed cropping season under different scenarios over the course of 15 years. Their simulations predict that Cry2A resistance evolution is maximized when single-protein varieties expressing Cry1A and two-protein varieties expressing Cry1A and Cry2A were both available. Cry2A resistance evolution is best managed when the introduction of two-protein varieties were early rather than late because initial Cry1A resistance allele frequencies increased with the delivery date. Cry1A resistance evolution is delayed when two-protein varieties expressing Cry1A and Cry2A and single-protein varieties expressing Cry2A

were available. That is, the introduction of the second protein, Cry2A, reduces the risk of resistance to Cry1A, but increases the risk of resistance to Cry2A. Cry2A and Cry1A resistance evolution was managed most effectively when single-protein varieties expressing these proteins were not commercially available. Their results suggest that two-protein minimum refuge requirements for Cry1A and Cry2Ab pyramided products may be lower than for each single-protein

Hurley (2000) performed a bioeconomic evaluation of the gradual introduction of different Bt corn products containing single or multiple Bt proteins over 30 years. The results demonstrate that adding a second high-dose protein to an existing high-dose or moderate-dose protein decreases the risk of resistance relative to a single high-dose protein or a single moderate-dose protein when the amount of refuge is identical. Adding a second high-dose protein to an existing high-dose protein provides the greatest protection. Evaluation of Bollgard II indicates that Cry2Ab is more effective in controlling TBW, CBW, and PBW than Cry1Ac. Hurley (2000) indicates that if the second protein is more effective, the decrease in resistance to the initial protein and the increase in resistance to the second protein are larger. Thus, extending this argument to Bollgard II, because Cry2Ab is more effective than Cry1Ac, the predicted durability of this stacked product will be somewhat less than if Cry2Ab and Cry1Ac were equally effective and both were expressed at a high dose to control TBW, CBW, and PBW. Still, the overall durability of Bollgard II will be greater than if Bollgard (Cry1Ac alone) or Bollgard II segregant (Cry2Ab segregant) were introduced sequentially or in a mosaic.

Both Livingston et al. (2002) and Hurley (2000) provide simulations that predict that adding a second protein to an existing single protein variety decreases the risk of resistance to the initial protein, while increasing the risk of resistance to the new protein. Their simulations also demonstrate that less refuge is necessary to preserve the same durability for a pyramided variety than for a single-protein variety. The results of both of these analyses indicate that rapid introduction of the stacked variety will not increase the risk of resistance and will likely delay resistance than would the sequential introduction of single proteins. They also demonstrate that the benefits of introducing a stacked variety of Bt cotton declines when the two proteins are not equally effective (both are not high dose), but are still higher than either single protein introduced sequentially.

4. Structured Refuge

Monsanto has proposed to incorporate the use of Bollgard II cotton into the currently required refuge options: 1) 5% external, unsprayed structured refuge (must be within ½ mile of Bollgard fields and at least for Bollgard cotton. These are: 150 feet wide, but preferably 300 feet wide), 2) 5% embedded refuge (must be at least 150 feet wide, but preferably 300 feet wide), 3) 20% external, sprayed structured refuge (must be within 1 mile of the Bollgard fields), and 4) community refuge (either 5% external, unsprayed or 20% external, sprayed refuge options allowed). The current refuge options for Bollgard cotton are discussed in detail in Section III, "Bt Cotton Confirmatory Data and Terms and Conditions of the Amendment", of EPA's recent Bt Crops Plant-Incorporated Protectant Biopesticides Registration Action Document (USEPA 2001).

Based on the modeling results discussed above, the currently required IRM program for Bollgard cotton is more than sufficient for Bollgard II. That is, all three refuge options are more protective against insect resistance for the three target pests, TBW, CBW, and PBW, using Bollgard II which expresses two insecticidal proteins, Cry2Ab2 and Cry1Ac, than for either Bollgard cotton expressing just the Cry1Ac protein or for a Bollgard II segregant expressing just the Cry2Ab2 protein. While a structured refuge is still necessary for pyramiding to be effective in delaying resistance, the size of the refuge may be smaller for the two proteins deployed in a pyramid (e.g., Bollgard II expressing both Cry1Ac and Cry2Ab2) to produce a similar delay when the two proteins are deployed sequentially (e.g., Bollgard cotton expressing only Cry1Ac and Bollgard II segregant expressing only Cry2Ab) (see discussion in Roush 1998). However, because both Bollgard II and Bollgard (and other Bt cotton varieties not yet commercialized) will both be deployed commercially for some overlapping period of time, potentially more than five years, it would be prudent, conservative, practical and provides growers a uniform message regarding IRM, for Bollgard II cotton and Bollgard cotton to have the same structured refuge requirements. In addition, until there is further evidence that other hosts are proven to be suitable, only non-Bt cotton should be relied upon as refuge.

5. Resistance Monitoring

Monsanto states that a Bollgard II monitoring plan will be developed as an extension of the current Bollgard monitoring plan for the TBW/CBW and PBW programs. Monsanto indicates that baseline susceptibility data to the Cry2Ab (specifically the Cry2Ab2) toxin for the key pests, TBW, CBW, and PBW, were being collected during the 2002 growing season at various locations across the Cotton Belt. Monsanto will submit an interim report on the 2002 Cry2Ab2 protein baseline data to EPA for review in 2003 (Arthur, 2002). Monsanto will continue to collect baseline data during the 2003 season and submit a final report to EPA in 2004. It is recommended that Monsanto provide the baseline susceptibility data for the Cry2Ab2 toxin for the 2002 and 2003 growing seasons, establish diagnostic concentrations for testing for resistance to Cry2Ab2, and provide a detailed resistance monitoring plan for both the Cry1Ac and Cry2Ab2 toxins. It is also recommended that the current resistance monitoring requirements mandated for Bollgard be mandated for Bollgard II (see USEPA 2001, see Section III. "Bt Cotton Confirmatory Data and Terms and Conditions of the Amendment" and Registration Decision Memorandum dated September 29, 2001 for the monitoring requirements).

The need for proactive resistance detection and monitoring is critical to the survival of Bt technology. For Bollgard, Monsanto is required to monitor for insect resistance (shifts in the frequency of resistance-conferring alleles) to the Bt toxins as an important early warning sign to resistance development in the field and to determine whether IRM strategies are working. An additional value of resistance monitoring is it may provide validation of parameters used in IRM models. Effective monitoring programs should have well-established baseline susceptibility data, sensitive detection methods, and a reliable collection network. Chances of finding resistant larvae in Bt cotton depend on level of pest pressure, frequency of resistant individuals, number of samples, and sensitivity of the detection technique. Therefore, as the frequency of resistant individuals or the number of collected samples increases, the likelihood of sampling a resistant individual increases (Roush and Miller 1986). The goal is to detect resistance in an insect

population before the occurrence of widespread crop failures, and if possible, in time so that mitigation practices can delay the development of resistance.

EPA has imposed specific monitoring requirements on Monsanto for its Cry1Ac plant-incorporated protectant as expressed in cotton (Bollgard™ cotton) (USEPA 2001, Section III). EPA has mandated that Monsanto will monitor for resistance and/or trends in increased tolerance for TBW, CBW, and PBW. There were approximately 5.7 million acres of Bollgard™ Bt cotton planted in the 2001 growing season and 4.5 million acres planted in the 2000 growing season (Monsanto 2002; USEPA 2001). It would be logistically and practically impossible to sample every farm that planted Bollgard™ (or in the future Bollgard II) cotton. Therefore, current resistance monitoring programs have focused sampling in areas of highest adoption of the Bt crops as the areas in which resistance risk is greatest.

For TBW and CBW, at least 20 specific collection sites will be established in time for the 2003 growing season. Sites must be focused in areas with high risk of resistance (e.g. where adoption is at least 75% of the cotton planted in that county or parish) while overall being distributed throughout the areas where TBW and CBW are important pests with a goal of having sites in AL, LA, AR, MS, FL, VA, GA, NC, SC, TN, and TX. For PBW, collection sites must be focused in areas of high adoption, with the goal of including all states where PBW is an economic pest (i.e., AZ, CA, NM, TX). There is a sampling goal stipulated to collect at least 250 individuals from any one location with a target of least 20 locations for TBW, CBW, and PBW. The greater the number of samples and number of locations, the greater the probability that resistant individuals will be collected.

The currently required, basic detection method has been a discriminating dose/diagnostic dose bioassay system that would distinguish between resistant and susceptible phenotypes, but such tests have been criticized as being too insensitive to be able to provide early detection before resistance develops or can spread very far, especially if the alleles for resistance are rare in the insect population. Discriminating dose bioassays are most useful when resistance is common (homozygous recessive alleles, i.e., field failure levels) or conferred by a dominant allele when the resistance allele frequency is greater than 0.01 (Andow and Alstad, 1998; Andow et al., 1998). It is currently considered as one of the central components of any monitoring plan, but other monitoring methods, such as the F_2 screen and DNA markers, may have value in conjunction with the discriminating concentration assay. Diagnostic concentration assays are already in use for the Cry1Ac toxin for testing for resistance development in TBW, CBW, and PBW. Monsanto recommends the use of diagnostic concentration assays to test for resistance development to the Cry2Ab toxin.

6. Remedial Action

EPA required a remedial action plan for Bollgard cotton be available in the unfortunate situation that resistance is suspected or actually does develop (USEPA 2001 and Registration Decision Memorandum dated September 29, 2001). These plans define not only suspected and confirmed resistance, but also the key steps and actions needed if and when resistance develops. The Arizona Bt Cotton Working Group has produced “A Remedial Action Plan for PBW Resistance

to Bt Cotton in Arizona" (see USEPA 2001, Appendix 1). An interim remedial action plan is currently required and is being revised to address TBW and CBW resistance to Bt cotton, key economic pests of cotton in the mid-South and Southeastern US (see USEPA 2001, Appendix 2). Monsanto has submitted to EPA a revised remedial action plan in May 2002 for Bollgard cotton to address TBW and CBW, but this plan has not yet been accepted. A key attribute of these plans is having the farmer's involvement in the plan's development.

Generally, if resistance is confirmed, the farmers involved will treat their Bt crop with alternative pest control measures. This might be a chemical pesticide known to be highly effective against the insect or it might mean measures such as crop destruction. In addition, the sales and distribution of the Bt crop would be suspended in that area and the surrounding area until it can be determined that insects in that area have regained their susceptibility to the Bt protein. There would also need to be increased monitoring to define the remedial action area(s). Other remedial action strategies include increasing refuge size, changing dispersal properties, use of sterile insects, or use of other modes of actions. Geospatial surveys would help define the scale of remedial action and where to intensify monitoring. Because no field resistance has yet been found, all of these tactics are untested.

Monsanto indicates that the basis of the Bollgard plan is appropriate for Bollgard II. However, because Cry1Ac and Cry2Ab protein are both expressed in Bollgard II there is a built-in resistance mitigation program and that the remedial action plan should only be implemented if a field population develops resistance to both the Cry1Ac and Cry2Ab proteins. If the idea is to protect the susceptibility of Bt (including Bt microbial formulations), then remedial actions should be considered and implemented if susceptibility to either Cry1Ac or Cry2Ab significantly changes. Thus, a more conservative remedial action program would consider the impact of susceptibility changes to either Cry1Ac or Cry2Ab and make appropriate modifications to the IRM program. However, these changes would need to involve the appropriate stakeholder groups, including EPA, prior to any institution of major remedial action measures.

7. Grower Education and Compliance

Grower education and compliance are central to the success of any IRM program. Monsanto has committed to implement comprehensive education programs that would be appropriate to convey the importance of complying with the IRM program to growers of both Bollgard and Bollgard II. A detailed discussion of Monsanto's education programs and the results of grower surveys (regarding compliance, data indicate greater than 91% with size requirements) for Bollgard (since 1996) are found in the Agency's Bt Plant-Incorporated Protectants Reassessment Document (USEPA 2001, Section IID.). The grower education requirements are described in this same document (USEPA 2001, see Section III. "Bt Cotton Confirmatory Data and Terms and Conditions of the Amendment" and the Registration Decision Memorandum dated September 29, 2001). Because of the importance of grower education, these same requirements are required for Bollgard II cotton.

Grower compliance with refuge and IRM requirements is a critical element for resistance management. Significant non-compliance with IRM among growers may increase the risk of resistance for Bt cotton. However, it is not known what level of grower non-compliance will compromise the risk protection of current refuge requirements. Therefore, in addition to carrying out an effective IRM education for growers, Monsanto must also establish a broad compliance program for Bollgard II just as it is required to do for Bollgard cotton. The current compliance program requirements are described in the Agency's Bt Plant-Incorporated Protectants Reassessment Document (USEPA 2001, see Section III. "Bt Cotton Confirmatory Data and Terms and Conditions of the Amendment" and the Registration Decision Memorandum dated September 29, 2001). Ideally, this compliance program would 1) establish an enforcement structure that will maximize compliance, 2) monitor level of compliance, and 3) investigate effects of noncompliance on IRM. Grower compliance with IRM strategies for Bollgard cotton (or any pesticide technology) is tied into the belief that new technologies, such as Bollgard II cotton (cotton expressing multiple Bt toxins (Cry2Ab and Cry1Ac), other new synthetic insecticides or other biological controls, will reduce the risk of resistance.

F. BENEFITS AND PUBLIC INTEREST FINDING

1. Seed Increase Registration Considerations

When deciding whether a pesticide meets the FIFRA section 3 standard for registration, EPA considers the risks and the benefits of a pesticide. See FIFRA section 3(c)(5), 3(c)(7)(A), (B), and (C); see also FIFRA section 2(bb). In addition, in order to grant a conditional registration under FIFRA section 3(c)(7)(C), EPA must also find that use of the pesticide is in the public interest. The benefits of a pesticide and the public interest assessment often overlap, at least to some degree. The following section describes EPA's benefits and public interest findings with respect to Monsanto Company's application for a seed increase registration.

a Criteria for Public Interest Finding

The criteria for a determination as to whether registration of a pesticide chemical is in the public interest are set forth in a 1986 Federal Register Notice entitled Conditional Registration of New Pesticides, 51 Fed. Reg. 7628 (Mar. 5, 1986). Thus, there is a presumption that registration of a pesticide is in the public interest if one of the following criteria is met: (i) the use is for a minor crop; (ii) the use is a replacement for another pesticide that is of continuing concern to the Agency; (iii) the use is one for which an emergency exemption under FIFRA Section 18 has been granted for lack of an alternative pest control method, or (iv) the use is against a pest of public health significance. Notwithstanding whether a registration of a pesticide chemical may be presumed to be in the public interest, EPA may determine that such a registration is in the public interest on the basis of the following criteria: (i) there is a need for the new chemical that is not being met by currently registered pesticides; (ii) the new pesticide is comparatively less risky to health or the environment than currently registered pesticides; or (iii) the benefits

(including economic benefits) from the use of the new active ingredient exceed those of alternative registered pesticides and other available non-chemical techniques.

Monsanto Company indicates that the target market for Bollgard II cotton is 45% of the U.S. cotton acreage that experiences consistent lepidopteran pest pressure. They intend to replace Bollgard cotton with Bollgard II cotton. They project that after five years following commercial introduction of Bollgard II cotton, approximately 80 percent of the Bollgard cotton acres will be replaced. Bollgard cotton acreage planted in 2001 was approximately 5.8 million acres (37% of the total Upland cotton acreage).

b Efficacy Benefits

Bollgard II cotton has significant efficacy benefits including improved performance (relative to Bollgard cotton) against cotton bollworm (CBW) and certain secondary pests including: soybean looper (SL), cabbage looper (CL), saltmarsh caterpillar (SMC), beet armyworm (BAW), and fall armyworm (FAW). Little additional efficacy benefits from use of Bollgard II cotton are expected for tobacco budworm (TBW) and pink bollworm (PBW). This is due to the fact that Bollgard cotton provides almost complete control of these pests and little or no insecticide is used on Bollgard cotton acreage specifically for TBW or PBW.

c Yield Benefits

Bollgard II cotton and Bollgard cotton have substantially similar yields (i.e., there is no statistical difference). Both Bollgard and Bollgard II cotton cultivars yielded better than the non-transgenic cultivar, DPL50. In 2002, the pests that are targeted to be controlled by Bollgard II cotton have reduced yield by 1.34 percent, which is $6/acre (at $442/acre). Thus, the primary benefit of Bollgard II cotton is saving the cost of an additional treatment (at $16/acre), while the secondary benefit is to potentially increase yield.

d Insect resistance management benefits

Bollgard II cotton has the potential to considerably increase the durability of either Cry2Ab and Cry1Ac as insect protection tools and offers a potentially more effective tool to delay resistance to the Cry1Ac and Cry2Ab proteins and increase the opportunities for integrated pest management. The use of Cry2Ab and Cry1Ac as a pyramid in Bollgard II cotton is considerably better for insect resistance management than the use of either Bt protein alone. Simulation models predict an approximately six-fold delay in the development of resistance with the Cry2Ab and Cry1Ac pyramid relative to the use of each protein sequentially (each single protein variety deployed one after another) (see U.S. EPA (2002)). The durability of Bollgard II cotton is driven by the Cry2Ab protein. Cry1Ac and Cry2Ab have different modes of action. Bollgard II cotton has been shown in the laboratory to have a functional high dose for TBW, CBW, and PBW (see U.S. EPA, 2002). Based on bioeconomic modeling simulations by Livingston et al. (2002) and Hurley (2000), introduction of Bollgard II cotton should be as swiftly as possible to maximize the resistance management benefits of both Cry2Ab and Cry1Ac in the pyramid. Since Cry2Ab has a different mode of action than Cry1Ac, Bollgard II cotton should remain

efficacious in the event that a pest species develops resistance to either of the single toxins (Cry1Ac or Cry2Ab).

e Economic benefits

The major economic benefits of Bollgard II cotton are that it will expand both the pest spectrum and life of the Bollgard technology. Based on Monsanto Company's projections (Monsanto, 2002), Bollgard II cotton is projected to displace eighty percent of Bollgard cotton within five years following initial commercialization. The present value of total U.S. benefits of Bollgard II cotton are estimated to exceed $12 million at a minimum to approximately $900 million, depending upon the discount rate used. This analysis is based on extending the life of the Bollgard technology from 10 to 25 years.

An assessment of annual grower benefits is based on the construction of demand curves for Bollgard cotton and Bollgard II cotton. Grower benefits are defined as the difference between the willingness to pay and the actual technology fee. The analysis of the two demand curves, and in particular the marginal revenue per acre of additional Bollgard II cotton, suggests that the technology fee would likely increase by approximately $5 per acre. For all growers, the gross benefit is $11.20 per acre and the net benefit is $5.24 per acre for Bollgard II cotton if the increased in technology fee is included. U.S. total annual net incremental benefits are predicted to be $43.8 million for Bollgard II cotton as compared to Bollgard cotton.

f Insecticide use reduction benefits

Use of Bollgard II cotton will result in some additional chemical insecticide use reduction and potential yield improvement. The gross benefits of $11.20 per acre will likely result from some combination of chemical savings ($16/acre is cost of average application) and yield improvement of $6 per acre (see Williams, 2002). Using the $43.8 million total annual net incremental benefits for Bollgard II cotton as compared to Bollgard cotton, this translates into a chemical saving of $50 million or 3.1 million acre treatments, which is approximately 14% of the 22.9 million acre treatments in 2001.

Although the exact amount cannot be quantified at this time, the Agency has previously documented the benefits and reduction in insecticide use for Bollgard cotton (see U.S. EPA 2001, Section E. "Benefits Assessment"). A qualitative analysis indicates that supplemental insecticidal applications for control of CBW will be further reduced and may be zero in many areas. However, the grower will still need to control for other insect pests such as plant bugs and stink bugs. Bollgard II cotton appears to produce a functional high dose for control of TBW, CBW, and PBW (see U.S. EPA, 2002). For the secondary pests, the greatest insecticide use reduction will be for soybean looper, beet armyworm, and fall armyworm. The exact amount of pesticide reduction will vary from year-to-year depending on the sporadic nature of these pests and other local conditions.

g Human health benefits

Human health protection will be further enhanced (over Bollgard cotton) by the additional insecticide use reduction benefits expected through the use of Bollgard II cotton.

h Environmental benefits

Environmental benefits will be further enhanced (over Bollgard cotton) by the additional insecticide use reduction benefits and indirect benefits expected through the use of Bollgard II cotton.

2. Full Commercial Section 3 Registration

Monsanto was granted a seed increase registration of 20,000 acres on June 14, 2002 (expiration June 14, 2003) to establish breeding nurseries and seed increase fields of Bollgard II event 15985 cotton. These seed increase acres were to be planted in Arizona (80 locations; 8,000 acres), Mississippi (32 locations; 11,700 acres) and Louisiana (3 locations; 300 acres). This review applies to the analysis of the benefits for Bollgard II cotton for a Section 3 full commercial registration. Bollgard II cotton expresses the *Bacillus thuringiensis* Cry2Ab2 protein pyramided with the already registered Cry1Ac protein (Cry1Ac is the protein found currently in Bollgard, Reg. No. 524-478).

Bollgard II cotton appears to produce a functional high dose for TBW, CBW, and PBW (see U.S. EPA, 2002). This means that Bollgard II has higher efficacy for CBW than Bollgard cotton. It also has an increased target spectrum for a number of secondary lepidopteran pests than Bollgard cotton. Bollgard II cotton is intended to protect cotton from feeding by tobacco budworm (*Heliothis virescens,* TBW), pink bollworm (*Pectinophora gossypiella*, PBW), cotton bollworm (*Helicoverpa zea*, CBW), cabbage looper (*Trichoplusia ni*, CL), saltmarsh caterpillar (*Estigmene acrea,* SC), cotton leaf perforator (*Bucculatrix thurbeiella,* CLP), soybean looper (*Pseudoplusia includens,* SL), beet armyworm (*Spodoptera exigua,* BAW), fall armyworm (*Spodoptera frugiperda*, FAW) and yellowstriped armyworm (*Spodoptera ornithogolli*, YSA).

a. Bollgard and Bollgard II Cotton Usage

Monsanto intends to work towards replacement of Bollgard cotton with Bollgard II cotton (Monsanto, 2002). Monsanto estimates that the target market for Bollgard II cotton would consist of approximately 45% of the U.S. cotton market that experiences consistent lepidopteran pressure. Theoretically, without a price barrier and no market competition, it would be possible to plant Bollgard II cotton on all 45% of the target market acres. They project that after five years following commercial introduction of Bollgard II cotton, approximately 80 percent of the Bollgard cotton acres will be replaced. Bollgard cotton acreage planted in 2001 was approximately 5.8 million acres (37% of the total Upland cotton acreage). This would mean that approximately 4.6 million acres would be expected to be planted with Bollgard II cotton within five years. A five year transition period is necessary because not all cotton production

areas will have high performing Bollgard II cotton varieties available initially. More importantly, growers will have to make choices based on yield performance of each variety balanced with the option of improved insect protection offered by Bollgard II cotton. A summary of the Bollgard cotton acreage by state is provided below in Table 1.

Table 1. Summary of Bollgard cotton acreage by state from 1996-2001

State	Bollgard™ Acreage Planted[1]					
	1996	1997	1998	1999	2000	2001
Alabama	348,810	251,784	306,535	398,683	314,500	255,777
Arizona	53,290	175,537	207,713	197,911	210,245	244,409
Arkansas	166,881	113,490	111,818	173,652	294,364	612,266
California	618	9,868	29,129	91,705	54,584	76,161
Florida	52,836	55,030	53,377	45,249	48,974	51,976
Georgia	375,744	533,340	508,842	693,288	580,908	568,087
Kansas	-	-	-	-	1,056	304
Kentucky						980
Louisiana	157,411	202,080	244,616	382,839	450,076	713,605
Mississippi	443,986	410,333	506,149	746,163	800,775	1,247,740
Missouri	498	592	519	6,254	21,415	88,448
New Mexico	393	2,693	20,869	12,263	12,242	16,393
North Carolina	20,519	21,027	77,490	274,312	424,880	543,888
Oklahoma	11,772	7,103	11,459	69,545	90,925	131,759
South Carolina	53,864	91,891	71,894	176,149	128,684	131,362
Tennessee	10,833	17,431	57,649	390,245	380,453	476,061
Texas	98,819	186,654	276,520	458,694	570,410	608,118
Virginia	86	37	1,876	6,300	24,857	36,490
U.S. Total	1,796,390	2,078,890	2,486,493	3,585,437	4,409,348	5,803,824

[1]Bollgard cotton acres are calculated on the bags of seed sold and the standard seed drop rate (recommended seed drop rate plus 15%) for the respective areas.

b. Efficacy of Bollgard II Cotton

A major benefit of transgenic cotton expressing Bt proteins is the possibility of reducing or eliminating conventional insecticide treatments for lepidopteran cotton pests. The degree to which insecticide use can be reduced is dependent on the efficacy of the transgenic Bt cotton against various target pests. Bollgard cotton (commercialized in 1996) expresses the Cry1Ac Bt toxin, which is active against certain lepidopteran cotton pests. Bollgard II cotton also expresses the Cry1Ac toxin at the same concentration as Bollgard cotton (approx. 10.0 µg/g dry weight) (Penn et al. 2001). Therefore, Bollgard II cotton should have similar efficacy against the target pests of Bollgard. However, since Bollgard II cotton also expresses the Cry2Ab toxin (> 10 fold more than Cry1Ac), which has a different mode of action than Cry1Ac, additional efficacy against secondary lepidopteran cotton pests may be observed. In fact, the efficacy observed from the combination of Cry1Ac and Cry2Ab in Bollgard II cotton may be greater than the efficacy observed with either of the toxins tested individually. The Agency has previously documented the benefits and reduction in insecticide use for Bollgard cotton (see U.S. EPA 2001, Section E. "Benefits Assessment"). This section will evaluate the efficacy of Bollgard II cotton (expressing both Cry1Ac and Cry2Ab) relative to Bollgard cotton against major and secondary cotton pests and the potential for reduction in insecticide use.

The insecticides used to control lepidopteran pests in conventionally grown (non-Bt) cotton consist mainly of organophosphates, pyrethroids, chlorinated hydrocarbons, and carbamates including: methyl parathion, cyfluthrin, acephate, cypermethrin, profenofos, esfenvalerate, thiodicarb, deltamethrin, tralomethrin, endosulfan, spinosad, methomyl, amitraz, and others. These pesticides have varying application rates and many are toxic to humans and non-target wildlife (U.S. EPA 2001, Table E.11).

The proposed label for Bollgard II cotton claims efficacy (control or suppression) of the following pests: tobacco budworm (*Heliothis virescens*, TBW), cotton bollworm (*Helicoverpa zea*, CBW), pink bollworm (*Pectinophora gossypiella*, PBW), cabbage looper (*Trichoplusia ni*, CL), saltmarsh caterpillar (*Estigmene acrea*, SMC), cotton leaf perforator (*Bucculatrix thurbeiella*, CLP), soybean looper (*Pseudoplusia includens*, SL), beet armyworm (*Spodoptera exigua*, BAW), fall armyworm (*Spodoptera frugiperda*, FAW), and yellowstriped armyworm (*Spodoptera ornithogolli*, YAW).

Efficacy Against Major Lepidopteran Cotton Pests

The major lepidopteran pests of cotton include tobacco budworm (TBW), cotton bollworm (CBW), and pink bollworm (PBW). There are multiple cotton production zones that have been identified in the U.S., each with different pest pressures. In general, TBW is the primary lepidopteran cotton pest in the mid south (i.e. Mississippi Delta), CBW in the southeast, and PBW in the far west (i.e. west Texas through California). Combined, TBW and CBW are the most damaging insect pests in cotton in the U.S. in 2001, infesting over 9 million acres (64% of the total U.S. crop) and requiring insecticide treatment on over 5 million acres (data taken from Williams 2002). PBW losses are restricted primarily to cotton growing regions in New Mexico and Arizona, where over 300,000 acres were infested in 2001. In Arizona, PBW were reported to have infested 99% of cotton acreage during the 2001 growing season (Williams 2002).

Bollgard

Bollgard cotton is known to express a "high dose" of Cry1Ac for both the TBW and PBW. A high dose is defined as 25 times the amount of toxin needed to kill susceptible larvae (see U.S. EPA 2001, section II.D. "Insect Resistance Management"). In theory, a high dose should represent nearly complete control of a target insect in the field, provided that the population has not developed resistance to the toxin. On the other hand, Bollgard cotton does not contain a low or moderate dose for CBW, meaning that suppression rather than complete control is likely in the field.

In terms of field data, Bollgard cotton has been shown to reduce the amount of insecticide used for TBW, CBW, and PBW in numerous studies. Monsanto has summarized multiple studies documenting insecticide reduction of 2.0 to 5.5 sprays per year with Bollgard in southern cotton growing regions (Monsanto 2001a). Data from Mississippi has shown that insecticide use (1996-1998) for TBW and CBW in Bollgard cotton ranged from 0.3 to 1.2 sprays/year as opposed to 3.1 to 5.2 sprays/year in non-Bollgard cotton (Hardee et al. 2001). However, cotton fields in the south typically require supplemental insecticide treatment for CBW. For example, in Louisiana, 1-3 applications/year in Bollgard cotton are still needed for CBW (although 4-8 applications/year are required in non-Bollgard cotton) (Leonard et al. 2001). In North Carolina, Bollgard cotton treated with pyrethroids (1-2 applications) for CBW has had significantly less boll damage and yield loss than untreated Bollgard cotton (Burd et al. 1999). According to crop loss data, over 2.7 million Bollgard cotton acres (~50% of all Bollgard cotton acres) were treated for CBW across the U.S. in 2001, typically averaging 1 to 1.6 applications (Williams 2002). For PBW, data from the 2001 growing season show that none of the Bollgard cotton acreage (189,000) planted in Arizona was treated for the pest. Conversely, of the non-Bollgard cotton acreage (89,000) in Arizona, over 53,000 acres were treated for PBW with an average of 1.3 applications (data taken from Williams 2002).

Bollgard II

Given the fact that Bollgard II cotton expresses essentially the same amount of Cry1Ac as Bollgard cotton, the efficacy of Bollgard II cotton against the major lepidopteran cotton pests should be at least as good as Bollgard cotton. However, Bollgard II cotton also expresses Cry2Ab, which is known to have activity against TBW, CBW, and PBW and may improve the overall efficacy of the product, particularly with CBW. A number of field studies have shown that this is in fact the case -- the efficacy of Bollgard II cotton is comparable or substantially better than Bollgard cotton.

For TBW, laboratory studies conducted with Bollgard cotton and Bollgard II cotton tissue showed that Bollgard II cotton had 3.5 fold more mean activity than Bollgard (Penn et al. 2001). Field studies conducted by Adamczyk et al. (2001) found that both that Bollgard cotton and Bollgard II cotton reduced TBW infestation by 99.9%. Drop cloth samples showed that Bollgard II cotton plots actually had fewer TBW larvae than Bollgard cotton

plots (0.0 vs. 0.25 per plot), although the difference was not statistically significant. Also, laboratory and greenhouse studies conducted with a Cry1Ac-resistant TBW colony showed little or no survival on transgenic cotton hybrids containing both Cry1Ac and Cry2Ab (Gould 2001).

For CBW, numerous studies have shown improved efficacy with Bollgard II cotton relative to Bollgard cotton. For example, the efficacy of Bollgard II cotton was evaluated in a series of field and greenhouse studies conducted in North Carolina during 2000 and 2001. In the 2000 study (Jackson et al. 2001), field trials showed that when compared with Bollgard cotton, Bollgard II cotton plants had fewer live CBW larvae present in terminal regions (0.25% vs. 0.67% of sampled plants), bolls (0.17% vs. 1.0%) and squares (0.0% vs. 0.42%), although the differences were not statistically significant. However, tissue damage was significantly less in all Bollgard II cotton plant tissues, in many cases at least 10 fold less than damage observed in Bollgard cotton. In greenhouse trials with Cry1Ac-tolerant and susceptible CBW strains, both Bollgard cotton and Bollgard II cotton plants had fewer surviving larvae and plant damage than conventional cotton, although there was no significant difference between the two. Field studies conducted in 2001 (Jackson et al. 2002) showed that Bollgard II cotton produced fewer CBW (156 pupae/acre, 156 adults/acre) than Bollgard cotton (518 pupae/acre, 298 adults/acre), although the differences were not significant. Supplemental insecticide treatments on Bollgard II cotton reduced CBW survival to zero and slightly reduced pest damage. Another set of field studies conducted in South Carolina also showed that significantly fewer CBW larvae were sampled in Bollgard II cotton plots than in Bollgard cotton (Ridge et al. 2001). Other field studies conducted in Mississippi showed significantly less CBW boll damage (0.13 damaged bolls/plot) and fewer larvae (0.0 larvae/plot) in Bollgard II cotton plots versus Bollgard cotton plots (2.25 damaged bolls/plot, 0.5 larvae/plot). Laboratory assays conducted as part of the same study generally showed less CBW feeding and survival on Bollgard II cotton than on Bollgard cotton, although the differences in many cases were not statistically significant (Stewart et al. 2000). In a second Mississippi field study, significantly fewer CBW larvae were found in Bollgard II cotton plots (8 total) versus Bollgard cotton plots (38 total) (Akin et al. 2001). Experiments performed in Texas also showed improved efficacy with Bollgard II cotton. Leaf disc tests in the laboratory revealed significantly higher CBW mortality on Bollgard II cotton leaf tissue after 10 days exposure (81.7 %) than Bollgard cotton (20.0 %) and lower larval weight among survivors (0.003 vs. 0.008 g). Likewise, larval counts in field plots showed no CBW larvae in Bollgard II cotton plots, significant fewer than the 4.4 larvae per plot found in Bollgard cotton plots (Norman and Sparks 2001).

Collectively, the efficacy results for CBW show that Bollgard II cotton has substantially improved activity relative to Bollgard cotton. Bollgard II cotton was shown to have consistently less CBW feeding damage and larval survival than Bollgard cotton, although in a number of cases the differences were not statistically significant. This improved efficacy may allow growers planting Bollgard II cotton to reduce the number of supplemental insecticide treatments currently needed with Bollgard cotton to fully control CBW (Ridge et al. 2001). However, given some of the studies showing even greater efficacy with CBW supplemental sprays on Bollgard II relative to unsprayed Bollgard II cotton (Jackson et al. 2001, 2002;

Stewart et al. 2000), it will be difficult to predict the exact amount of insecticide use that will be reduced until Bollgard II cotton is adopted on wide scale by growers.

For PBW, like TBW, Bollgard cotton provides near total control. Therefore, Bollgard II cotton should perform as well or better, considering both events express similar amounts of Cry1Ac and Bollgard II cotton also expresses high levels of Cry2Ab. This assumption has been supported by field data developed in Arizona (Marchosky et al. 2001). An analysis of sampled cotton bolls showed that Bollgard cotton and Bollgard II cotton had between 0.0 and 3.83% PBW-infested bolls compared with 47.5 to 93.43% PBW-infested bolls for conventional cotton. The authors also noted that significantly fewer viable larvae were recovered from Bollgard cotton and Bollgard II cotton PBW-infested bolls than from conventional PBW-infested bolls, presumably due to early instar death of larvae in Bollgard cotton bolls (larvae were killed by Bt toxins soon after infestation). In general, Bollgard II cotton bolls showed less infestation than Bollgard cotton bolls, although the differences were not statistically significant. Larval sampling of Bollgard II cotton revealed fewer older larvae (> than first instar) than in Bollgard cotton (which also had few older larvae), indicating very quick control of larval infestations. Overall, the authors concluded that Bollgard II cotton obtained 99% PBW suppression, an order of magnitude higher than the already very effective Bollgard cotton.

Efficacy Against Minor Lepidopteran Cotton Pests

The secondary lepidopteran pests of cotton include: cabbage looper (CL), saltmarsh caterpillar (SMC), cotton leaf perforator (CLP), soybean looper (SL), beet armyworm (BAW), and fall armyworm (FAW) and European corn borer (ECB). The label for Bollgard II claims efficacy against all of these pests (and also the yellowstriped armyworm, YAW). These pests generally occur sporadically and infest fewer acres in cotton growing regions than the major cotton pests. However, as conditions warrant, they may inflict significant economic losses and require insecticide treatment. Combined, these secondary pests can account for considerable insecticide use in cotton.

According to crop. loss data from the 2001 growing season (taken from Williams 2002), infestations and insecticide treatments for secondary cotton pests were as follows (acres have been rounded): 1) CL: 1,080,000 acres infested (7 % of the total U.S. cotton crop) with 122,000 acres treated; 2) SMC: 2,030,000 acres infested (14 % of all cotton) with 405,000 acres treated; 3) CLP: 150,000 acres infested (1% of all cotton) with 9,000 acres treated; 4) SL: 830,000 acres infested (6 % of all cotton) with 289,000 acres treated; 5) BAW: 3,270,000 acres infested (22 % of all cotton) with 301,000 acres treated; 6) FAW: 640,000 acres infested (4 % of all cotton) with 61,000 acres treated. Data were not available for YAW.

Bollgard

Monsanto does not specifically claim activity with Bollgard cotton against any of the secondary pests listed above (source: Monsanto 2001 Technology Use Guide). Additionally,

data submitted by Monsanto shows that Cry1Ac has little activity against either FAW or BAW (Monsanto 2001b). However, Cry1Ac has been shown to have some activity against SL (Monsanto 2001b) and CL (MacIntosh et al. 1990). Despite the activity of Cry1Ac against some secondary cotton pests, it is unclear whether Bollgard cotton has had an impact in the need for control or amount of insecticide used to manage these pests. It is noted that based on crop loss data collected from 1996 - 2000, no change in the secondary status of these pests has been observed nationally or regionally.

Bollgard II

Data submitted by Monsanto has shown that Cry2Ab has more activity than Cry1Ac against FAW and BAW and equivalent activity against SL (Monsanto 2001b). Other secondary pests (such as SMC, CL, and CLP) were not specifically evaluated by Monsanto. However, there have been a number of field and laboratory studies that have evaluated the efficacy of Bollgard II cotton against secondary cotton pests.

For CL, a larval survey in Texas using drop cloth samples of a test field was conducted to determine pest pressure from a number of species. The results showed that Bollgard II cotton plots contained no CL larvae, while both Bollgard cotton (63.3 larvae/6 foot row) and conventional cotton (54.6) contained significantly higher pest numbers (Norman and Sparks 2001).

For SMC, a field test conducted in Mississippi using drop cloth sampling for larvae found that Bollgard II cotton plots had greatly reduced SMC infestation (0.25 larvae/plot) relative to Bollgard cotton (2.75) and conventional cotton (8.75) (Adamczyk et al. 2001). However, the differences were not significantly different and additional study may be needed to fully evaluate efficacy against SMC.

For SL, a number of studies have demonstrated high efficacy from Bollgard II cotton. Samples from a Mississippi field test plot showed that Bollgard II cotton significantly reduced SL larvae (0.25 larvae/plot) relative to Bollgard cotton (37.75 larvae/plot) or conventional cotton (32.0 larvae/plot) (Adamczyk et al. 2001). A second Mississippi field study showed similar results -- Bollgard II cotton plots had significantly fewer total SL larvae (17) than Bollgard cotton (389) or conventional cotton (276) (Akin et al. 2001). These trends were also observed in a third Mississippi study - Bollgard II cotton (1.3 larvae/row), Bollgard cotton (36.2 larvae/row) and conventional cotton (19.3 larvae/row) (Stewart et al. 2000) - and a South Carolina study (Ridge et al. 2001). Collectively, these results demonstrate that a high level of SL control is possible with Bollgard II cotton, while Bollgard cotton had relatively little effect on SL populations.

For BAW, high efficacy has also been observed with Bollgard II cotton. A laboratory assay using leaf disc tissue showed significantly higher BAW mortality with Bollgard II cotton (73.3 to 100%) relative to Bollgard cotton (8.3 to 47.5%) and conventional cotton (0 to 27.5%). A related larval sampling of field plots showed no BAW larvae in Bollgard II cotton plots, compared with 1.6 larvae/plot in Bollgard cotton and 2.3 larvae/plot in conventional

cotton (Norman and Sparks 2001). Larval sampling in Mississippi showed fewer BAW in Bollgard II cotton plots (2.5/plot) than Bollgard cotton (35.5/plot) or conventional cotton (42.0/plot) (Adamczyk et al. 2001). An analysis of BAW larvae on plant tissue, showed significantly less feeding on Bollgard II cotton tissue relative to Bollgard cotton or conventional tissue. (Stewart et al. 2000).

For FAW, high efficacy of Bollgard II cotton has also been shown in a series of studies. A laboratory study showed that of FAW larvae fed Bollgard II cotton leaf tissue, none survived to pupation. However, survival on Bollgard II cotton blooms was higher, although somewhat less than FAW fed Bollgard cotton or conventional cotton blooms (Stewart et al. 2000). In a Mississippi field study, the total number of sampled FAW larvae was lowest in Bollgard II cotton plots (1) versus Bollgard cotton (24) and conventional cotton (28) (Akin et al. 2001). A second Mississippi study revealed the same trend: Bollgard II cotton (0.75 larvae/plot), Bollgard cotton (1.75 larvae/plot), and conventional cotton (3.0 larvae/plot) (Adamczyk et al. 2001). A third study in Texas also showed similar patterns: Bollgard II cotton (0 larvae/row), Bollgard cotton (4.4 larvae/row), and conventional cotton (4.3 larvae/row) (Norman and Sparks 2001).

Efficacy Benefits Summary for Bollgard II Cotton

A previous analysis by the Agency (U.S. EPA 2001) has demonstrated the reduction in insecticide use resulting from the efficacy Bollgard cotton against major cotton pests (TBW, CBW, and PBW). Since Bollgard II cotton has been shown to be as efficacious as Bollgard against these target pests (both express the same amounts of the Cry1Ac protein), these benefits should be maintained with the use of Bollgard II cotton. In addition, Bollgard II cotton, because of the expression of Cry2Ab, has a number of other significant efficacy benefits including improved performance (relative to Bollgard cotton) against CBW and certain secondary pests including: SL, CL, SMC, BAW, and FAW.

For TBW and PBW, there will likely be little additional efficacy benefits from use of Bollgard II cotton. This is due to the fact that Bollgard cotton provides almost complete control of these pests and little or no insecticide is used on Bollgard acreage specifically for TBW or PBW. Also, since Cry2Ab has a different mode of action than Cry1Ac, the product should remain efficacious in the event that a pest species develops resistance to either of the single toxins (Cry1Ac or Cry2Ab).

The greatest efficacy benefit of Bollgard II cotton may be with CBW. For CBW, many growers employing Bollgard cotton typically need supplemental sprays to control feeding damage and increase yield. According to 2001 crop loss data, approximately 50% of Bollgard cotton acreage in the U.S. was treated for CBW. Depending on economic thresholds for cotton production, the efficacy of Bollgard II cotton will likely reduce (and may eliminate) the need for supplemental CBW treatments. However, the exact amount of potential insecticide reduction will be difficult to quantify prior to widespread use of Bollgard II cotton and will be dependent on pest pressure and other local conditions.

For the secondary pests, Bollgard II cotton efficacy was greatest against CL (one study), SMC (one study), SL, BAW, and FAW. Given the lack of comprehensive data for several pests (CL, SMC), additional study may be needed to fully assess the efficacy of Bollgard II cotton against these pests. Also, no data were available to determine Bollgard II cotton efficacy against CLP or YAW, which are claimed as target pests on the product label. In terms of the potential to reduce insecticide use, the benefits from Bollgard II cotton will likely be greatest (based on the available data) with SL, BAW, and FAW, as Bollgard cotton has limited or no efficacy against these pests. However, since these insects are secondary (occasional) pests of cotton, the exact amount of pesticide reduction will likely be limited (no individual secondary pest was treated on more than 405,000 acres in 2001) and will vary year-to-year depending on pest pressure and other local conditions. The efficacy benefits for Bollgard II cotton are summarized in Table 2 below.

Table 2. Efficacy benefits for Bollgard II cotton

Pest	Bollgard II Efficacy - Potential Benefits Relative to Bollgard
TBW	Comparable or better efficacy than Bollgard.[1]
CBW	Improved efficacy relative to Bollgard, with the potential to reduce or eliminate some insecticide treatments. However, may not completely eliminate the need for supplemental insecticide use for total control.
PBW	Comparable or better efficacy than Bollgard.[1]
CL	Complete control was observed in a single field study (much greater efficacy than Bollgard).[2]
SMC	Greater efficacy than Bollgard observed in a single field study, although not statistically significant.[2]
CLP	No data - efficacy unknown
SL	High efficacy (Bollgard has no efficacy against SL). Potential to reduce some insecticide use.
BAW	High efficacy (Bollgard has no efficacy against BAW). Potential to reduce some insecticide use.
FAW	High efficacy (Bollgard has no efficacy against FAW). Potential to reduce some insecticide use.
YAW	No data - efficacy unknown

[1] Because nearly complete control of these pests is achieved with Bollgard cotton, there will not likely be any additional benefit resulting from Bollgard II cotton.

[2] Given the lack of comprehensive studies or statistical significance, more data may be needed to fully determine the efficacy of Bollgard II cotton against these pests.

c. Yield benefits

Bollgard II cotton and Bollgard cotton have substantially similar yields in Arizona for PBW control, but there is additional control of Citrus Peelminer (Marchosky et al., 2001). In Mississippi, yields for Bollgard II cotton and Bollgard cotton are essentially equal; however, in these experimental plots, there was superior control for soybean looper, beet armyworm, fall armyworm, and cotton bollworm (Akin et al. 2001; Stewart et al., 2000). In all studies, both Bollgard and Bollgard II cotton cultivars yielded better than the non-transgenic cultivar, DPL50. Bollgard II cotton is expected to be superior to Bollgard cotton based on its efficacy for soybean looper, beet armyworm, fall armyworm because Bollgard cotton has limited or no efficacy on these pests (see efficacy discussion above). In 2002, the pests that are targeted to be controlled by Bollgard II cotton have reduced yield by 1.34 percent, which is $6/acre (at $442/acre) (see table 3 below and Williams 2002). Thus, the primary benefit of Bollgard II is saving the cost of an additional treatment (at $15/acre), while the secondary benefit is to potentially increase yield.

Table 3. Percent yield reduction from targeted pests of Bollgard II cotton

Pest	% infested	% treated	average % yield reduction
Corn Borer	0.2%	1.7%	0.000
Cotton Leafperforator	1.0%	5.9%	0.000
Pink Bollworm	2.1%	27.0%	0.039
Fall Armyworm	4.1%	9.6%	0.022
Soybean Loopers	5.4%	34.9%	0.008
Cabbage Loopers	7.0%	11.3%	0.000
Salt-marsh Caterpillars	13.1%	20.0%	0.013
Beet Armyworm	21.1%	9.2%	0.031
Bollworm/budworm	60.7%	54.8%	1.228
		Yield for all affected pests	1.341

Source: Williams 2002. Cotton Insect losses, 2001.

d. Insect resistance management benefits

Bollgard II cotton producing two different insect control proteins (Cry2Ab and Cry1Ac) with different modes of action in a pyramid, in combination with a refuge and other components of an insect resistance management plan, could delay the development of insect resistance by approximately six-fold as compared to the use of either protein alone. Bollgard II cotton has better efficacy against CBW, and an increased target spectrum for several secondary pests. It has been shown to produce a functional high dose for control of TBW, CBW, and PBW (see U.S. EPA, 2002). Based on simulations using pyramids, Bollgard II cotton is better at delaying resistance using all currently mandated refuge options, including the riskiest, 5% external, unsprayed refuge option, than Bollgard cotton. EPA's review of the likelihood of insect resistance development and insect resistance management for Bollgard II cotton in U.S. EPA (2002). The bottom-line conclusion is that Bollgard II cotton offers the potential to extend the duration of the use of Bt proteins for insect pest control by providing a potentially effective tool

to delay resistance to the Cry1Ac and Cry2Ab proteins and increased opportunities for better integrated pest management programs. Described below is a brief summary of the insect resistance management benefits of Bollgard II cotton caused by the pyramiding of the *cry1Ac* and *cry2Ab* genes.

How quickly the insect resistance management benefits of Bollgard II cotton are realized will depend upon the speed of its introduction. It is expected that some overlap among Bollgard cotton (one gene = Cry1Ac), Bollgard II cotton (two genes = Cry2Ab and Cry1Ac) and potentially, other transgenic Bt cotton varieties containing these Bt genes or others will occur in the next five or more years. Monsanto notes that it intends to work towards replacement of Bollgard cotton with Bollgard II cotton (Monsanto, 2002). They project that after five years following commercial introduction of Bollgard II cotton, approximately 80 percent of the Bollgard cotton acres will be replaced. Bollgard cotton acreage planted in 2001 was approximately 5.8 million acres (37% of the total Upland cotton acreage, see Table 1).

Both Livingston et al. (2002) and Hurley (2000) provide bioeconomic simulations that predict that adding a second protein (Cry2Ab) to an existing single protein variety (Bollgard cotton expresses the Cry1Ac protein) decreases the risk of resistance to the initial protein, while increasing the risk of resistance to the new protein. Their simulations also demonstrate that less refuge is necessary to preserve the same durability for a pyramided variety than for a single-protein variety. The results of both of these analyses indicate that rapid introduction of the pyramided variety, Bollgard II cotton, will not increase the risk of resistance and will likely delay resistance several fold more than that of the sequential introduction of the single proteins. They also demonstrate that the benefits of introducing a pyramided variety of Bt cotton declines when the two proteins are not equally effective (both are not high dose), but are still higher than either single protein introduced sequentially.

e. Economic benefits

Economic benefits summary

The major economic benefits of Bollgard II cotton are that it will expand both the pest spectrum and life of the Bollgard technology. Based on Monsanto Company's projections (see Monsanto, 2002), Bollgard II cotton is expected to displace Bollgard cotton. Eighty percent of Bollgard cotton is expected to be replaced by Bollgard II cotton within five years following initial commercialization. Aggregate benefits of Bollgard II cotton are estimated to have a present value of between $130 million and $1.4 billion if the technology is extended for 10 to 25 years, using either a 40% discount rate or a 7% discount rate, respectively. Based on profit maximizing behavior, results of the demand curve suggest that the technology fee for Bollgard II cotton may need to be approximately $5 more, on average, than for Bollgard cotton. The annual net benefits per acre (using the $5 increase in technology fee) are $5.24 for Bollgard II cotton as compared to Bollgard cotton. The annual net incremental benefits are $43.8 million for Bollgard II cotton.

Aggregate benefits

Aggregate benefits are computed on a present value basis since the main benefit is to extend the life of the technology from 10 to 25 additional years. The discount rate is critical to this analysis. Factors that go into the rate are the cost of capital for the government and the uncertainty with which the anticipated benefits will be realized. This report uses two discounts rates, 7% and 40%, to represent reasonable limits for low to high uncertainty. The 7% discount rate represents the Office of Management and Budget rate (see Circular No. A-94, Transmittal Memo No. 64, October 29, 1992), a relatively risk-free rate of return similar to that assumed for a long-term Treasury bond. The 40% discount rate represented a highly speculative rate of return similar to that assumed for venture capitalists. The discount rate is defined as the interest rate used in calculating the present value expected yearly for benefits and costs. There are likely differing views on the appropriate discount rate to use. The factors leading to the uncertainty are new commercial pest control products that will also mitigate Bt resistance and whether the existing refuge, resistance monitoring, and other IRM requirements alone will be sufficient to prevent resistance from occurring. Based on these two discount rates, the aggregate benefits of Bollgard II cotton are estimated to have a present value of between $130 million and $950 million if the technology is extended for 10 years, using the 40% discount rate and the 7% discount rate, respectively (see Table 4 below). If the technology is extended for 25 years, the aggregate benefits will be $130 million to $1.4 billion, using the 40% discount rate and the 7% discount rate, respectively.

Table 4. Summary of aggregate benefits

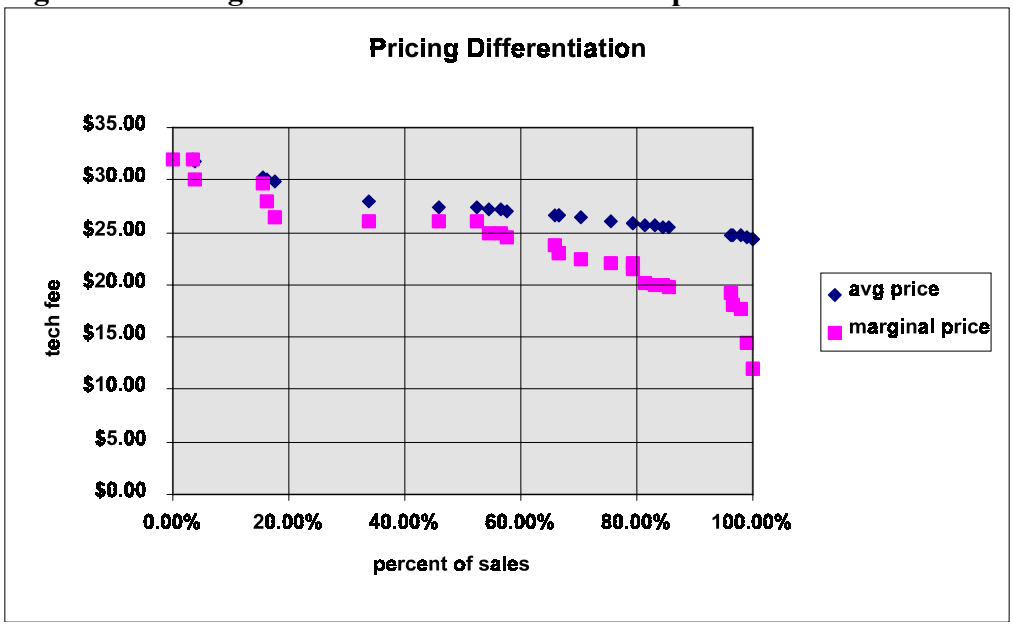
Discount rate assumed	**Extending life of Bt technology using Bollgard II cotton (millions of $)**	
	10 years	**25 years**
40 percent	$129.54	$130.52
7 percent	$950.49	$1,404.84

The benefits associated with extending the life of the Bollgard II cotton technology will be achieved by all growers who use the technology. The aggregate benefits to growers may be overstated to the extent that pricing differentiation can charge more when the individual benefits are higher. Because the Bt trait is added to a hybrid which is optimized for a particular area, pricing differentials for the technology fee are practical and expected. This has been the case for Bollgard cotton. Uncertainties associated with this analysis include the discount rate, the technology fee, field performance, new technologies obviating the need for Bollgard II cotton, and the likelihood of resistance.

Pricing differentiation based on technology fees and level of adoption

Figure 1 below shows that 80% of the projected sales of Bollgard II cotton seed would have a technology fee priced between $22 and $32 per acre. It is important to note that EPA, not the Monsanto Company, has chosen the range of technology fees that might be used in different areas of the Cotton Belt. In addition to pricing behaviors, other forms of the demand curve could change these findings. This analysis is therefore speculative, but suggests that the demand curve for Bollgard II cotton will be influenced by the technology fee and the anticipated need for pest control just as it was for Bollgard cotton. For the major markets, the technology fee of Bollgard cotton currently varies between $20 to $32 per acre (Williams, 2002). Differential pricing permits a higher price when the demand is higher and will reduce the grower benefits estimated by a single model.

Figure 1. Pricing differentiation and level of adoption



The pricing differentiation analysis is derived from the cotton losses data on Bt costs (Williams, 2002). A detailed assessment of the demand profile for Bollgard II cotton should be detailed for each submarket on a regional basis. An aggregate demand curve would be the sum of each individual demand curve. Such an analysis was not done because it misrepresents the accuracy of demand curve assessment and the level of resolution. The purpose of the crude demand curve analysis is to do a rough cut (back of the envelope) assessment.

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Crude demand curves for Bollgard cotton and Bollgard II cotton

For the crude demand curves, EPA has estimated that the total potential Bollgard II cotton acres will be 10.5 million acres (based on Williams 2002, see Table 4). Likewise, EPA has estimated

that the total potential Bollgard cotton acres will be 9.8 million acres. Therefore, it is expected that Bollgard II cotton will add 700,000 acres to market size. The market size represents all acres that are infested with the lepidopteran pests claimed on the Bollgard II cotton draft label. The actual treated acres will be some proportion of the total potential infested acres. Also assumed is that the maximum benefits for Bollgard II cotton are $95 per acre and the Bt costs will be $20 per acre. For Bollgard cotton, the maximum benefits are $79 per acre and the Bt costs are $20 per acre. The maximum benefit is the highest willingness to pay in terms of potential insecticide costs. The maximum Bt cost is the cost associated with the technology, e.g., refuge, marketability, lower hybrid performance etc. The technology fee for Bollgard cotton is assumed to be $24.43 and for Bollgard II cotton, it is assumed to be $30.

The marginal revenue is defined as the difference in total revenue divided by the difference in total output from one year to the next. The demand curve for Bollgard cotton (Table 5) shows a marginal revenue of 0 between $30 and 35 per acre technology fee. However, the demand curve for Bollgard II cotton (Table 6) shows a marginal revenue of 0 between $35 to $40 per acre technology fee. The demand curve is based on a 20% improvement for the grower with the highest benefits primarily based on the expanded pest spectrum (see discussion of efficacy above). This equates to a cost of $16 per acre, the amount of an additional treatment for beet armyworm, for example (one of the pests that would be included for Bollgard II cotton, but not Bollgard cotton). Based on profit maximizing behavior, results of these demand curves suggest the technology fee may need to be increased to about $5 per acre for Bollgard II cotton. For a grower not needing the additional benefits of a broader pest spectrum of Bollgard II cotton, the increased price would not necessarily pay off, particularly in the short run.

Table 5. Crude demand curve for Bollgard cotton

Technology fee schedule ($)	Acres adopted (millions)	Total revenue (millions $)	Marginal revenue (millions $)	Marginal revenue per acre (million $)
75	0.10	7.35		
65	0.59	38.22	30.87	63.00
55	1.76	97.02	58.80	50.00
45	2.94	132.30	35.28	30.00
40	3.63	145.04	12.74	18.57
35	4.21	147.49	2.45	4.17
30	4.80	144.06	(3.43)	(5.83)
25	5.49	137.20	(6.86)	(10.00)
15	6.76	101.43	(35.77)	(28.08)
0[1]	8.62	0.00	(101.43)	(54.47)

[1]There will be less than 100% adoption of Bollgard cotton even if there is no technology fee. Here it is 88% adoption.

Table 6. Crude demand curve for Bollgard II cotton

Technology fee schedule ($)	Acres adopted (millions)	Total revenue (millions $)	Marginal revenue (millions $)	Marginal revenue per acre (million $)
75	1.16	86.63		
65	2.10	136.50	49.88	52.78
55	3.26	179.03	42.53	36.82
45	4.31	193.70	14.70	14.00
40	5.04	201.60	7.88	10.71
35	5.57	194.78	(6.82)	(13.00)
30	5.99	179.55	(15.23)	(36.25)
25	6.83	170.63	(8.92)	(10.62)
15	7.88	118.13	(52.50)	(50.00)
0[1]	9.45	0.00	(118.13)	(75.00)

[1]There will be less than 100% adoption of Bollgard II cotton even if there is no technology fee. Here it is 90% adoption.

Annual benefits of Bollgard cotton and Bollgard II cotton

The annual benefits of Bollgard cotton are $124.9 million annually. While the benefits of Bollgard II cotton are $168.6 million annually. The incremental benefit is $5.24 per acre and the net annual incremental benefit is $43.8 million per year for Bollgard II cotton as compared to Bollgard cotton. This information is summarized in Table 7 below. Even if the average technology fee for Bollgard II cotton were $5 higher than Bollgard cotton, the average benefits for Bollgard II cotton are projected to be $27.63 per acre. The average benefits for Bollgard cotton are projected to be $22.39 per acre. The net benefits are calculated to be $5.29 per acre for Bollgard II cotton.

Table 7. Annual benefits of Bollgard cotton and Bollgard II cotton
(assume technology fee of $25 for Bollgard cotton and $30 for Bollgard II cotton)

	Cotton acres adopted (millions)	**Per acre net benefit ($)**	**Annual benefits (millions $)**
Bollgard cotton	5.58	22.39	124.9
Bollgard II cotton	6.10	27.63	168.6
Incremental benefits		5.24	43.8

f. Insecticide Use Reduction

Reducing the use of pesticides more toxic than Bt will reduce the risks to human health and the environment. Numerous studies have demonstrated an overall reduction in insecticide sprays for lepidopteran pests (TBW, CBW, and PBW) as a result of the introduction of Bollgard cotton in the U. S. (USEPA, 2001, Table E.13; Monsanto, 2001a, Table 1). Based on cotton insect loss data from 1991-2000, the three primary pests, TBW, CBW, and PBW, account for more than 77% of the yield lost and 84% of the insecticide use due to lepidopteran infestation in cotton. The major chemical insecticides used on cotton are: methyl parathion, cyfluthrin, acephate, cypermethrin, profenofos, esfenvalerate, thiodicarb, deltamethrin, tralomethrin, endosulfan, spinosad, methomyl, amitraz (USEPA, 2001; Table E.11).

Bollgard II cotton is expected to reduce insecticide use further because of its increased efficacy against CBW and increased secondary insect spectrum which includes armyworms, loopers, saltmarsh caterpillar (see efficacy discussion above). This additional insecticide use reduction cannot be quantified. Bollgard II cotton has been shown in the laboratory to have a functional high dose for CBW (see U.S. EPA, 2002; Monsanto, 2001b).

A qualitative analysis of preliminary field studies indicates that supplemental insecticidal applications used for control of CBW when using Bollgard cotton will likely not be needed when using Bollgard II cotton (Layton and Long, 2001; Ridge et al. 2001). In most cases, this means that the current average of 1.6 supplemental (primarily pyrethroid) treatments for CBW in Bollgard cotton fields will be essentially zero. However, the grower will still need to control for other insect pests such as plant bugs and stink bugs (Monsanto, 2002). For the secondary pests, the potential to reduce insecticide use will be the greatest for soybean looper, beet armyworm, and fall armyworm. The exact amount of pesticide reduction will likely be limited since no individual secondary pest was treated on more than 405,000 acres in 2001 (Williams, 2002) and will vary from year-to-year depending on the sporadic nature of these pests and other local conditions. Bollgard II cotton will likely replace some of the use of indoxacarb and methoxyfenocide that Bollgard cotton does not as well as reduce the use of pyrethroids and spinosad (Monsanto, 2002). No additional insecticide use reduction is expected for Bollgard II cotton for control of TBW and PBW since Bollgard cotton provides essentially complete control for these pests.

Use of Bollgard II cotton will result in some additional chemical insecticide use reduction and potential yield improvement. The gross benefits of $11.20 per acre will likely result from some combination of chemical savings ($16/acre is cost of average application) and yield improvement of $6 per acre (see Williams, 2002). Using the $43.8 million total annual net incremental benefits for Bollgard II cotton as compared to Bollgard cotton, this translates into a chemical saving of $50 million or 3.1 million acre treatments, which is approximately 14% of the 22.9 million acre treatments in 2001.

g. Human health benefits

Human health protection will be further enhanced by the additional insecticide use reduction benefits expected through the use of Bollgard II cotton. The human health benefits for Bollgard cotton have been previously summarized by EPA (USEPA, 2001, Section II.E.) and are discussed in Monsanto Company's public interest document (Monsanto, 2001a). These benefits include farm worker safety and bystander protection.

h. Environmental benefits

Non-target organisms

Non-target organism protection will be further enhanced by the additional insecticide use reduction benefits expected through the use of Bollgard II cotton. The non-target organism benefits for Bollgard cotton have been previously summarized by EPA (USEPA, 2001, Section II.E.).

Indirect benefits

Indirect benefits associated with Bollgard cotton will be further enhanced using Bollgard II cotton. These include increased effectiveness of beneficial arthropods as pest control agents, reduction in potential impacts to wildlife, reduced risk from pesticide-run-off, reduced fuel usage, and lower levels of air pollution and related waste production. For example, elimination of pyrethroid sprays for CBW and preserving beneficial insects with the use of Bollgard II cotton will likely reduce the requirement for spray application for aphids (Monsanto, 2002). In addition, Monsanto notes that fuel consumption savings computed based on approximately 5 million acres of Bollgard cotton planted in the U.S. in 2000 were approximately $410,000 or approximately 456,000 gallons of fuel.

III. Data Gaps

1. Avian Oral

2. Ladybeetle beetle larval toxicity. A newly required dietary toxicity study should be conducted to determine the NOEC for the Ladybeetle beetle larvae.

IV. Regulatory Position

A. Existing Seed Increase Registration

Pursuant to FIFRA section 3(c)(7)(C), EPA may conditionally register a pesticide containing a new active ingredient if: 1) insufficient time has elapsed since the imposition of the data requirement for those data to be developed and all other required data have been submitted, 2) the use of the pesticide product during the period of the conditional registration will not cause any unreasonable adverse effect on the environment, and 3) the registration and use of the pesticide during the conditional registration is in the public interest. BPPD believes that all these criteria have been fulfilled.

For the Bollgard II seed increase registration, which contains two active ingredients, one of which has not previously been registered (Cry2Ab2), the first criterion under FIFRA section 3(c)(7)(C) mentioned above has been met. Insufficient time has elapsed since the imposition of the following data requirements: Avian Oral and Lady Beetle. In October of 2000 the SAP recommended that, for a final risk assessment, studies that strengthen the hazard analysis are needed. EPA agreed with this recommendation and in its *Bt* Crop Reassessment of October 2001 required that avian subchronic studies be generated by the *Bt* <u>corn</u> registrants. A 10% cottonseed meal in the diet is not representative of all poultry feeds. Thus, BPPD is recommending imposing a requirement to repeat this avian study with higher levels of the Cry2Ab2 protein. Because BPPD does not believe that this data requirement was reasonably foreseeable by the applicant, BPPD is recommending granting a conditional registration while such study is being conducted.

As for the Lady Beetle requirement, a dietary toxicity study should be required to determine the NOEC for lady beetle larvae. As EPA has not previously required such a lady beetle *larvae* study for other registered PIP products, BPPD does not believe that the applicant would reasonably have foreseen this data requirement. For this reason, BPPD is recommending granting a conditional registration while such study is being conducted.

The applicant has submitted or cited data to satisfy the second criterion for conditional registration under FIFRA 3(c)(7)(C) as mentioned above. Monsanto submitted and/or cited satisfactory data pertaining to the proposed use (seed increase). The human health effects data and non-target organism effects data are considered sufficient for the period of the conditional registration (1 year). These data demonstrate that no foreseeable human health hazards or ecological effects are likely to arise from the use of the product and, under the terms and conditions of the registration, the risk of resistance developing to *Bacillus thuringiensis* during the conditional registration is not expected to be significant because of the limited acreage involved. The data also demonstrate that, under the terms and conditions of registration (e.g., geographical limitations), there is virtually no possibility of any risk associated with weediness or outcrossing to wild relatives.

Registration of this plant-incorporated protectant in cotton is in the public interest as required by the third criterion because the benefits (including economic benefits) from the use of the new active ingredient exceed those of alternative registered pesticides and other available non-chemical techniques. In addition EPA believes that the new plant-incorporated protectant is comparatively less risky to health or the environment than currently registered pesticides other than the currently registered Bollgard and the *Bt* microbial pesticides.

In view of these minimal risks and the benefits, BPPD believes that the use of the product during the limited period of the conditional registration will not cause any unreasonable adverse effects and the seed increase registration is in the public interest.

Although the data with respect to this particular plant-incorporated protectant containing a new active ingredient are satisfactory, they are not sufficient to support an unconditional registration under FIFRA 3(c)(5). Additional data are necessary to evaluate the risk posed by the continued

use of this product. Consequently, BPPD recommends imposing the data requirements specified above in the attached Biopesticide Registration Action Document in Section III.

B. Amendment To Seed Increase Registration

Pursuant to Section 3(C)(7)(B), the Administrator may conditionally amend the registration of a pesticide to permit additional uses of such pesticide notwithstanding that data concerning the pesticide may be insufficient to support an unconditional amendment, if the Administrator determines that (i) the applicant has submitted satisfactory data pertaining to the proposed additional use, and (ii) amending the registration in the manner proposed by the applicant would not significantly increase the risk of any unreasonable adverse effect on the environment. If the applicant is unable to submit an item of data (other than data pertaining to the proposed additional use) because it has not yet been generated, the Administrator may amend the registration under such conditions as will require the submission of such data not later than the time such data are required to be submitted with respect to similar pesticides already registered under FIFRA.

Criterion (i) in the first paragraph has been met because the applicant has submitted all data pertaining to the proposed new (commercial) use of the product, including the incremental risks that would result from approval of the application, for the time period and under the terms and conditions for which the registration amendment is being considered. The human health effects data and non-target organism effects data are considered complete for a wide-scale full commercial use and no potential adverse effects are foreseen in these areas. The environmental effects data are considered complete for the time period and under the terms and conditions for which the registration amendment is being considered. Although the soil degradation that was required for unlimited full scale commercial use has been submitted, sufficient time has not passed for the Agency to have reviewed these data; in the May 2002 seed increase registration decision document, however, EPA noted that the soil degradation study was not necessary for registration as the seed increase registration was limited to a small acreage and for a limited time. [USEPA/BPPD, June 2002. Biopesticides Registration Action Document, Bacillus thuringiensis Cry2Ab2 protein and its genetic material necessary for its production in cotton, at 36.] Under the applicant's original seed increase registration, natural disaster (hurricane) destroyed all Bollgard II seed crops planted in the states of Mississippi and Louisiana leaving the applicant (registrant) with only 8,000 acres in the state of Arizona. Consequently, the acreage that can be planted for the next two growing seasons from these seeds is necessarily limited.[1] Accordingly, because this amendment is being granted for a relatively short time (approximately 17 months which is two growing seasons), and because the amount of acreage that can possibly be planted during this time period is minimal, BPPD believes that the soil degradation study is not necessary to support this short term and *de facto* limited acreage commercial use amendment. Finally, BPPD believes that the registrant has provided sufficient data to characterize the incremental risks associated with the development of resistance. The registrant has agreed to appropriate conditions and limitations, including refuge requirements, of the use of the product to mitigate these risks. In conclusion, amending the existing registration by accepting the new use amendment proposed by Monsanto Company would not significantly increase the risk of any unreasonable adverse effect on man or the environment. Although the

data with respect to this particular new use for the time period and under the terms and conditions being considered in the amendment are satisfactory, an acceptable soil degradation study would be necessary for an unlimited full-scale commercial registration and/or registration amendment. As discussed above in section II.D.2, the introduction of these products for a wide-scale use and for an unlimited period of time poses a potential risk to soil organisms**.** An acceptable study regarding this issue is necessary to determine whether this pesticide poses such a risk and, if it does pose a risk, whether such risk would be significant.

BPPD also believes that criterion (ii) under paragraph one has also been met because it appears that the proposed additional use does not "significantly increase the risk of any unreasonable adverse effect." In essence, FIFRA requires a determination that the proposed additional use of the product differs from the current use only in ways that would not modify the risk/benefit ratio so as to cause unreasonable adverse effects, taking into account the costs and benefits of the additional use as restricted by the terms and conditions of registration.[1]

As discussed in section II.E., the proposed new use of this product on greater acreage poses the risk of the development of resistance in certain pests of cotton. As a result, pests could develop resistance to certain microbial Bt pesticides as well as PIP Bt products that are applied to cotton and other crops. Microbial *Bt* pesticides are critical for many organic programs and are identified by the Agency as a safer pest control method than many chemical insecticide alternatives. The Agency further recognizes that microbial *Bt* pesticides have low dietary, worker, and ecological risks when compared to the more hazardous alternatives that might replace the microbial *Bt* pesticides should resistance develop. The microbial *Bt* pesticides also are important components in many IPM programs for a variety of crops and the loss of such pesticides could cause growers to substitute more harmful pest control agents. Resistance would significantly reduce the utility of such products.
As discussed in detail in section II.F. of this document, the proposed new use should provide substantial benefits to cotton producers, including continued and/or sustained reduction in conventional insecticide use, improved performance (relative to Bollgard cotton) against CBW and certain secondary pests, likely increased yields, and increased delay in resistance as compared to Bollgard. Because conventional insecticides are generally more toxic and environmentally hazardous, reducing the use of such pesticides will also reduce the risks to human health, including risks to farm workers and bystanders, and to the environment, such as to non-targets as well as other indirect effects.

The risks from pesticide resistance are substantial and BPPD has concluded the risks, if unchecked, could outweigh the benefits of the proposed new use. However, the terms and conditions of registration that are recommended below in this BRAD (requiring specific plans

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[1] The Agency has essentially already made a determination under the second prong of the statutory standard, i.e, that there is no "human dietary risk from residues that result from a use of a pesticide in or on any food inconsistent with the standard under section 408 of the [FFCDA]," FIFRA section 2(bb)(2), up until May 1, 2004, which is the date the temporary tolerance exemption for residues of Cry2Ab2 expires.

for refugia, monitoring, and compliance) during the period of the recommended registration will mitigate the risks from pesticide resistance sufficiently so that the risks of the proposed amended registration would not significantly increase the risks of unreasonable adverse effects. Furthermore, because there is only a limited amount of seed available the Agency does not believe that the registrant will have an amount of seed, that when planted, would significantly increase the risks of any unreasonable adverse effects on the environment, during the period of the proposed amendment.[2]

The temporary tolerance exemption for Cry2Ab2 is due to expire May 1, 2004. Moreover, addition to this commercial use amendment request, the registrant has requested an extension of time for the seed increase registration to allow them to submit and EPA to review two required studies set forth under the condition of the seed increase registration. These two studies are also required to support the 3(c)(7)(C) new use amendment. Taking all these considerations into account, BPPD believes it is appropriate to grant the seed increase registration and commercial use amendment for the same duration of time and for both of them to expire on or before the expiration date of the temporary tolerance exemption. Regarding the request for an extension of time for the expiration date of the conditional registration, upon further consideration, BPPD believes that the original date for submission of the avian oral and ladybeetle larval studies was too short for the registrant to adequately perform, analyze and submit the studies to the Agency. Therefore, BPPD recommends extending the deadline for the submission of those studies until June 15, 2003. Further, BPPD recommends extending the time period for the registration until May 1, 2004 to provide sufficient time for the Agency to review these studies and also to coincide with the expiration of the exemption from the requirement of a temporary tolerance. In view of the minimal risks as described in the initial seed increase registration as well as the benefits described therein, BPPD believes that the use of the product during this additional limited period of the conditional seed increase registration will similarly not cause any unreasonable adverse effects on the environment and that the seed increase registration is in the public interest. Regarding the request for an amendment for a new use, for the reasons indicated above, BPPD recommends granting a short term commercial use amendment under section 3(c)(7)(B) to the underlying seed increase registration. In as much as BPPD recommends extending the underlying seed increase registration until May 1, 2004, and the exemption from the requirement of a temporary tolerance is already set to expire May 1, 2004, BPPD believes it would be appropriate for the commercial use amendment to expire on the same date. BPPD has determined that the applicant has submitted satisfactory data pertaining to this proposed additional use (short term commercial use) and that amending the seed increase registration to allow for commercial use for the next 17 months, with the terms and conditions of the registration and especially in light of the *de facto* acreage limitation, would not significantly increase the risk of any unreasonable adverse effect on the environment. Accordingly, BPPD recommends that this time limited registration and the new use amendment be granted until 1 May, 2004.

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[2] Seed needed for planting cotton is 30 pounds/acre. The company had 8000 acres and harvested approximately 240,000 pounds of cotton seed. With the limited number of seed available, BPPD feels there will be minimal risks associated with this amendment to the seed increase registration.

V. Actions Required by Registrant

Conditionally required data must be submitted, and reports of incidences of adverse effects to humans or domestic animals and target pest resistance must be submitted under FIFRA, Section 6(a)2.

VI. Terms and Conditions of Amended Registration

- Submission of a 6 week Avian Oral Study at higher dosage

- Submission of Lady Beetle Larval Toxicity Study

- Expires May 1, 2004 at which time the time limited exemption from the requirement of a tolerance also expires.

- The following terms and conditions also apply. Note that the deadlines for submission of Cry1Ac data are the same deadlines as have been imposed for similar pesticides already registered.

  a) An analytical method for the detection of Cry2Ab2 protein in cotton and a thorough characterization of the antisera used in the method(s). The method must be validated by an independent laboratory validation and must be submitted on or before March 15, 2003.

  b) Amino acid sequence data submitted indicate that there are no similarities between Cry1Ac protein and any known toxins or allergens. However, the analyses submitted are not equivalent to a stepwise 8 amino acid analysis of the subject protein against available databases. These additional data are required to augment the health effects database for Cry1Ac cotton and must be submitted on or before March 15, 2003.

  c) Protein expression data in terms of dry weight, as the amount of protein present in the given tissue. Tissues for which expression data must be provided include: leaf, root, pollen, boll, seed, and whole plant. In addition, data for each of these tissues should be provided for young plants in rapid growth, plants during flowering, and mature plants before harvest when that part of the plant is present. Data are due on or before March 15, 2003.

  d) The Agency is requiring testing on accumulation and persistence of Cry1Ac protein under a range of conditions typical of *Bt* cotton cultivation. EPA requires Monsanto submit a test protocol before the studies are actually conducted. In general, the Agency anticipates that soils would be sampled from fields where *Bt* cotton has been grown for at least 3 years compared with fields where no *Bt* crop has been grown. These paired fields would be several locations through the cotton growing area of the US representing different soil and climatic variations. The Agency anticipates that samples would need to be taken 2 or 3 times during the growing season. Monsanto is required to submit a final report on January 31, 2004.

e) Confirmatory field data for possible impacts on non-target insects. Either existing studies must be submitted or Monsanto must submit final studies on or before January 31, 2004.

f) Field experiments on north-south movement of *Helicoverpa zea* from corn-growing regions to cotton-growing regions using radioisotope decay or other suitable methods. The study is due January 31, 2004.

g) Research on whether alternate hosts of *Helicoverpa zea* provide an effective refuge for *Bt* cotton. Research topics must include, but are not limited to, mating and oviposition behavior of *Helicoverpa zea*, fitness of adults and adult population densities coming from the alternate hosts vs unsprayed and sprayed *Bt* cotton, whether insect pest emergence is in synchrony with pests emerging from *Bt* cotton, the proximity of alternate hosts to *Bt* cotton, and refinement or construction of new resistance management models that include alternate hosts appropriate for different cotton production regions, e.g., North Carolina v. Louisiana. Studies must be conducted across the cotton belt where cotton bollworm is an economic pest. The sites must represent a range of conditions that will affect cotton bollworm biology. Conditions must include such factors as irrigation, soil types, and climatic conditions. An interim report is due March 15, 2003, and final report due March 15, 2004.

h) Research studies to determine the IRM value of different insecticide chemistries likely to be used against the cotton bollworm in conventional and transgenic *Bt* cotton (irrigated and non-irrigated, side by side field trials). Any potential effects must be related to survival of putative *Bt*-resistant cotton bollworm and effective refuge size. Usage data must be provided for insecticide use on *Bt* cotton fields from 1997 to 2001. Once this information has been gathered, Monsanto must refine or construct new resistance management models for appropriate cotton producing areas in the US (i.e., areas where *Helicoverpa zea* typically exceeds economic threshold on *Bt* cotton). Resistance management models must include consideration of supplemental insecticidal treatments for control of cotton bollworm. An interim report is due March 15, 2003, and final report due March 15, 2004.

Gene Flow

The following information regarding commercial production must be included in the grower guide for Bollgard® II Cotton and is a term of this amendment:

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

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

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

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

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

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

Insect Resistance Management

The required IRM program for *Bt* cotton must have the following elements:

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

2. Requirements for Monsanto to prepare and require *B*t cotton users to sign "grower agreements" which impose binding contractual obligations on the grower to comply with the refuge requirements;

3. Requirements for Monsanto to develop, implement, and report to EPA on programs to educate growers about IRM requirements;

4. Requirements for Monsanto to develop, implement, and report to EPA on programs to evaluate and promote growers' compliance with IRM requirements;

5. Requirements for Monsanto to develop, implement, and report to EPA on programs to evaluate whether there are statistically significant and biologically relevant changes in susceptibility to Cry1Ac protein in the target insects;

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

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

a. Refuge Requirements

All growers of *Bt* cotton must employ one of the following structured refuge options:

_Bt_ Cry2Ab2 Bollgard II Cotton Registration Action Document

1) External, Unsprayed Refuge

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

2) External Sprayed Refuge

Ensure that at least 20 acres of non-*Bt* cotton are planted as a refuge for every 80 acres of *Bt* cotton (total of 100A). The variety of cotton planted in the refuge must be comparable to *Bt* cotton, especially in the maturity date, and the refuge must be managed (e.g., planting time, use of fertilizer, weed control, irrigation, termination, and management of other pests) similarly to *Bt* cotton. The non-*Bt* cotton may be treated with sterile insects, insecticides (excluding foliar *Btk* products), or pheromones labeled for control of the tobacco budworm, cotton bollworm, or pink bollworm. Ensure that a non-*Bt* refuge is maintained within at least 1 linear mile (preferably within ½ mile or closer) from the *Bt* cotton fields.

3) Embedded Refuge

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

4) Embedded Refuge for Pink Bollworm Only

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

5) Optional Community Refuge Pilot

This option allows multiple growers to manage refuge for external, unsprayed and external, sprayed refuge options or both. This option is not allowed for the embedded/in-field options. A community refuge program will be allowed as a continuing pilot for the 2003 growing season. EPA will evaluate the community refuge program following the 2003 growing season. The community refuge for insect resistance management must meet the requirements of either the 5% external unsprayed refuge and/or the 20% sprayed option, or an appropriate combination of the two options. Monsanto must implement the 2003 community refuge pilot program as described in the Bollgard II Cotton 2003 Refuge Guide and perform the following actions. The community refuge pilot must consist of the following:

> There will be a community refuge coordinator for each pilot site. Each community refuge coordinator must submit a signed community refuge form listing all of the participants at the pilot site to Monsanto by May 31, 2003. Monsanto must provide EPA with a copy of the signed form and the community refuge coordinator will maintain a copy of the field map (to scale) or suitable scalar representation of the community refuge for review by Monsanto or EPA as part of the compliance program.
>
> Monsanto must conduct two phone audits of a statistically representative sample of community refuge coordinators from communities in all states participating in the community refuge. The phone audit shall occur no later than June 30, 2003. EPA shall review the questions prior to each phone audit.
>
> The community refuge program users must be included in telephone compliance survey and the on-farm visits to be conducted by Monsanto under section 3.c. below.
>
> Monsanto must provide a written report to EPA at the end of the 2003 growing season on community refuge use and compliance (due by January 31, 2004).
>
> Monsanto must conduct a review of the community refuge program and submit that review to the Agency as to any proposed changes by January 31, 2004. An appropriate amendment for any proposed changes must be submitted to the Agency.

At the request of Monsanto and based on EPA's review of the results of the 2001 community refuge pilot program, the requirements for the 2003 pilot program may be modified.

b. Grower Agreements

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

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

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

3) Monsanto must establish by the 2003 growing season, a system which is reasonably likely to assure that persons purchasing the *Bt* cotton product will affirm annually that they are contractually bound to comply with the requirements of the IRM program. The proposed system will be submitted to EPA on or before March 15, 2003.

4) Monsanto must continue to use its current grower agreement. If Monsanto wishes to change any part of the grower agreement that would affect either the content of the IRM program or the legal enforceability of the provisions of the agreement relating to the IRM program, thirty days prior to implementing a proposed change, Monsanto must submit to EPA the text of such changes to ensure the agreement is consistent with the terms and conditions of this amendment.

5) Monsanto must establish a system which is reasonably likely to assure that persons purchasing the *Bt* cotton sign grower agreement(s).

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

7) Beginning on January 31, 2004 and annually thereafter, Monsanto shall provide EPA with a report on the number of units of the *Bt* cotton seed shipped and not returned and the number of such units that were sold to persons who have signed grower agreements.

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

c. IRM Education and IRM Compliance Monitoring Programs

Monsanto must implement the following IRM education and compliance monitoring programs:

1) Monsanto must design and implement a comprehensive, ongoing IRM education program designed to convey to *Bt* cotton users the importance of complying with the IRM program. The program shall include information encouraging *Bt* cotton users to pursue optional elements of the IRM program relating to refuge configuration and proximity to *Bt* cotton fields. The education

program shall involve the use of multiple media, e.g. face-to-face meetings, mailing written materials, and electronic communications such as by internet or television commercials. Copies of the materials, including the Grower Guide, must be submitted to EPA for their records. The program shall involve at least one written communication annually to each Bollgard II cotton grower separate from the grower agreement. Monsanto shall coordinate its education program with educational efforts of other organizations, such as the National Cotton Council and state extension programs.

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

3) Beginning January 31, 2004 and annually thereafter, Monsanto shall provide a report to EPA summarizing the activities it carried out under its education program for the prior year and its plans for its education program during the current year.

4) Monsanto shall design and implement an ongoing IRM compliance assurance program designed to evaluate the extent to which growers are complying with the IRM program and that takes such actions as are reasonably needed to assure that growers who have not complied with the program either do so in the future or lose their access to the *Bt* cotton product. Monsanto shall prepare and submit by March 15, 2003 a written description of its compliance assurance program. Other required features of the program are described in paragraphs 5 - 12 below.

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

6) The IRM compliance assurance program shall include an annual survey of a statistically representative sample of *Bt* cotton growers conducted by an independent third party. The survey shall measure the degree of compliance with the IRM program by growers in different regions of the country and consider the potential impact of non-response. Monsanto shall provide a written summary of the results of the prior year's survey to EPA by January 31 of each year. Monsanto shall confer with EPA on the design and content of the survey prior to its implementation.

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

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

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

10) If a grower, who purchases *Bt* cotton for planting, was specifically identified as not being in compliance during the previous year, Monsanto shall visit the grower and evaluate whether that the grower is in compliance with the IRM program for the current year.

11) Beginning January 31, 2004 and annually thereafter, Monsanto shall provide a report to EPA summarizing the activities it carried out under its compliance assurance program for the prior year and its plans for its compliance assurance program during the current year. Included in that report will be the percent of growers using each refuge option (or combination of options) by region, the approximate number or percent of growers visited on farm by Monsanto, the number of tips investigated, the percent of growers who were not complying with the IRM requirements, and the follow-up actions taken.

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

d. Insect Resistance Monitoring.

The registration of Cry1Ac and Cry 2Ab2/Cry1Ac PIPs expressed in cotton is conditioned on Monsanto carrying out appropriate programs to detect the emergence of insect resistance as early as possible. Resistance monitoring programs include: surveying insects for potential resistance and collection of information from growers about events that may indicate resistance. The Agency is imposing the following conditions:

1) Monsanto shall provide a description to EPA of its resistance monitoring plan for Bollgard II by March 15, 2003. The description shall include: sampling (number of locations and samples per locations), sampling methodology, bioassay methodology, standardization procedures, detection technique and sensitivity, and the statistical analysis of the probability of detecting resistance. Monsanto shall provide to EPA the baseline susceptibility data for the Cry2Ab2 toxin for the 2003 growing season and establish diagnostic concentrations for testing for resistance to Cry 2Ab2 by January 31, 2004. Collection sites must be focused in areas of high adoption for pink bollworm, tobacco budworm, and/or cotton bollworm.

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

3) A report on results of resistance monitoring and investigations of damage reports must be submitted to the Agency annually by April 30th each year for the duration of the conditional registration.

e. Remedial Action Plans

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

1) Remedial Action Plan for Pink Bollworm

If resistance involves the pink bollworm (*Pectinophora gossypiella*), Monsanto must implement a modified Arizona *Bt* Cotton Working Group's Remedial Action Plan to include both Cry1Ac and Cry2Ab2 proteins. Monsanto must obtain approval from EPA before modifying the Arizona *Bt* Cotton Working Group's Remedial Action Strategy. The Arizona *Bt* Cotton Working Group's Remedial Action Plan for Cry1Ac can be found in Enclosure 1.

2) Interim Remedial Action Plan for Tobacco Budworm and Cotton Bollworm

A Remedial Action Plan for cotton bollworm and tobacco budworm must be developed and implemented by Monsanto if suspected or confirmed resistance is found to Cry1Ac and or Cry2Ab2 proteins. The Interim Remedial Action Plan for Cotton Bollworm and Tobacco Budworm is contained in Enclosure 2. After consultation with cotton growers and academic experts, Monsanto plans to submit a revised Remedial Action Plan by January 31, 2003 for EPA's review and approval. Monsanto must obtain approval from EPA before modifying the Remedial Action Plan for Cotton Bollworm and Tobacco Budworm.

Annual Reports

Monsanto will provide an annual report to EPA on its Cry1Ac and Cry 2Ab2/ Cry1Ac PIPs expressed in cotton. This report must include, but is not limited to, annual sales by county and by state (summed by state), research status for any outstanding data requirements as covered in 3

above, grower education completed last year and planned for the following year, the description of grower agreements in place, grower compliance with IRM requirements, use and compliance with the community refuge option, and insect resistance monitoring results.

This section 3 registration is subject to cancellation under section 6(e) of the Federal Insecticide, Fungicide and Rodenticide Act, as amended, if the company (Monsanto Company) does not comply with the terms and conditions of the registration.

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