

III. *Bt* Cotton Confirmatory Data and Terms and Conditions of the Amendment

A. *Bt* Cotton Confirmatory Data

The Agency has considered the data base available on Cry1Ac PIP expressed in cotton in light of our scientific review, the Scientific Advisory Panel (SAP) report of March 2001, and the public comments received. The Agency has made the determination that some additional data are needed to characterize better certain potential impacts from the continued use of this product. The following describes the data needed and the time frame for submission of these data.

1. Residue Analytical Methods

Analytical methods and method validation are not typically required for active ingredients where a tolerance exemption has been granted. However, these data are useful for determining whether or not the protein is expressed in the cotton plant and international bodies such as the Codex Alimentarius Commission (Codex) are gathering the validated methods for products of biotechnology that can be found in food. The Agency and the Federal Food and Drug Administration have also recently found value in having validated analytical methods for Plant-incorporated Protectants. EPA has guidelines for producing and validating analytical methods (under OPPTS Guidelines OPPTS 860.1340). These guidelines call for development of the method, validation by an independent laboratory, and validation by EPA before being accepted. For the protein in Cry1Ac cotton analytical method, it is also necessary to include a thorough characterization of the antisera used in the method(s). The analytical method and independent laboratory validation must be submitted to EPA by June 1, 2002 and EPA intends to complete its validation within one year of the registrant submission.

2. Protein Expression Data

EPA requested guidance from the SAP in December 1999 on the appropriate method to test for and report the amount of pesticidal protein in PIPs. Their recommendation to EPA was that the amount of pesticidal protein in a tissue be provided as either total protein or dry weight of the plant tissue and indicating the value of each type of data. The Panel went on to explain the value of collecting these data at different plant growth states in order to do appropriate toxicological testing. (SAP Report No. 99-06. February 4, 2000.) [Characterization and Non-Target Organism Data Requirements for Protein Plant-Pesticides. SAP Report No. 99-06. February 4, 2000. 49 pp.]

The Agency has received protein expression data for Cry1Ac and found it acceptable for the initial registration. However, data are not available for all types of tissues and the Agency agrees with the SAP that all of the expression data should be in a consistent format for all of the *Bt* crop products. These supplementary data must be determined and presented, 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, during flowering, and mature plants before harvest when that part of the plant is present. Data are due on or before March 15, 2003.

3. Amino Acid and DNA Sequence Data

Amino acid sequence data have been submitted and reviewed (MRID number 431452-01). These data were found acceptable, but since this time the value of comparing the amino acid sequence to known toxins and allergens has been highlighted and generally supported by the Scientific Advisory Panel. Data for the Cry1Ac protein which compares the amino acid sequence of the Cry1Ac protein expressed in cotton with known toxins and allergens has been submitted to the Agency has been evaluated. Based upon the submitted sequence comparison data, there does not appear to be any significant amino acid sequence similarity between the Cry1Ac (*Btk* HD-73) protein and known protein allergens and toxins. Significant sequence similarity is defined as 35% amino acid identity over an 80 amino acid contiguous sequence (Report of the WHO-FAO Expert Consultation, January 22-25, 2001, Rome, Italy). 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. These data must be submitted on or before March 15, 2003.

The Agency has done a preliminary review of additional DNA characterization data submitted during the month of August 2001 using genome walking, PCR, cosmid libraries, DNA sequencing, and Southern blotting. EPA's preliminary review of the additional DNA characterization data does not indicate a need for a change in the Agency's risk assessment. If EPA's final assessment of these data finds the data to be inadequate, the Agency will require further clarification or studies from the registrant under FIFRA section 3(c)(2)(B). In addition, the registrant is encouraged to use new methods (e.g. MALDI-TOFF), as they are validated to confirm amino acid sequence of the expressed protein more precisely.

4. Determination of Cry Protein Levels in Soils Following Several Years of *Bt* PIPs

In the March 12, 2001 SAP Report No. 2000-07 on *Bt* Plant-Pesticides Risk and Benefit Assessment, the October 2000 Scientific Advisory Panel (SAP) concluded that published data at that time did not adequately address the persistence of Cry proteins from *Bt* crops in the soil. Since it is difficult to correlate the relevance of the published laboratory studies to field situations, the SAP recommended field studies be conducted in established *Bt* fields in a variety of soil types and climatic conditions. The SAP suggested amount, accumulation and persistence of biological activity of Cry proteins in the soil are areas that should be investigated. EPA agrees with the SAP that actual field data on Cry1Ac protein levels in soil will yield relevant data on persistence and natural variation of *Bt* proteins in soil. If high levels of Cry1Ac protein

are found in field soils, reevaluation of the risks to certain non-target organisms might be required. Therefore, EPA is requiring additional supplementary studies regarding Cry protein in soil.

The Agency is requiring testing of Cry1Ac protein under a range of conditions typical of *Bt* cotton cultivation. EPA requires the registrant 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. The registrant is required to submit a protocol on or before March 15, 2002 with an interim report on January 31, 2003, and a final report on January 31, 2004.

5. Non-target Insects

In the February 7, 2000 report from the Scientific Advisory Panel meeting of December 8, 1999, the SAP responded to a question from EPA on field scouting to supplement acute testing of a few indicator insect species. The SAP stated:

Field scouting is an important tool to risk assessment, but should not replace Tier 1 testing. Only a limited number of species can be tested in laboratory bioassays, but field studies can be used to detail the impacts on species appropriate for the [PIP] being tested and in a manner that is relevant to determining ecological impacts. It is important that the conclusions drawn from the field studies be scientifically sound and not just correlative and that it reflect actual exposure to the [PIP]. . . Since ecological effects are critical to safety issues addressed by the Agency proposed rules, it would appear that field studies be included in the decision packet.

Such field studies were not required by EPA for the original registration decision for Cry1Ac in 1995. EPA is now requiring confirmatory field data for possible impacts on non-target insects. Either existing studies must be submitted or the registrant must submit a protocol for field survey studies on or before March 15, 2002 and final studies submitted on or before January 31, 2005.

6. Insect Resistance Management

a. North/South Movement of *Helicoverpa zea*

Helicoverpa zea is known as cotton bollworm when attacking cotton and corn earworm when attacking corn. It has other common names for some of its other host plants. *Helicoverpa zea* can have several generations per year and frequently the insect moves from corn to cotton. There is

not a high dose of the Cry proteins for *Helicoverpa zea* in either *Bt* corn or *Bt* cotton. If *Helicoverpa zea* survives exposure to *Bt* corn and then moves to *Bt* cotton, then the chances of resistance development are increased through the added exposure.

The October 2000 SAP indicated that there was more evidence of cotton bollworm (*Helicoverpa zea*) migration from the north to the south than evidence against this migration pattern. The Panel went on to discuss how the movement of cotton bollworm from the north to the south could impact insect resistance management, specifically refuge size. The Panel stated that as long as the amount of *Bt* corn in a (northern) region did not exceed 50%, then the refuge size was adequate. However, there are several areas in the Corn Belt where market penetration of *Bt* corn exceeds 50%. The registrant is required to conduct field experiments on north-south movement of *Helicoverpa zea* from corn-growing regions to cotton-growing regions using radioisotope decay or other suitable methods.

b. Alternate Hosts as an Effective Refuge and Insecticide Sprays on *Bt* Cotton

The SAP meeting held in 1998 and 2000 concluded that only non-*Bt* cotton could be used as an effective refuge until more data are gathered regarding alternate hosts as effective refuges. As part of their public comments to the “Draft Potential Risk Mitigation and Regulatory Options” paper of July 17, 2001, the registrant has provided the Agency with their analysis of crop plants grown near cotton serving as an additional refuge for cotton bollworm (*Helicoverpa zea*). The Agency has reviewed the data presented on crops (such as soybeans and sorghum) and weeds potentially serving as an additional refuge. While the Agency agrees that these crops are hosts for these insects and that adult moths of pest species emerge from soybeans and other crop and weed plants, data are not available to be sure that the timing of adult insect emergence and distribution of insects on each alternate host (distribution may be sporadic), density, fitness of adults emerging from other crops, and spatial arrangement of the planting areas for other crops are adequate to ensure that an effective refuge is present.

In order to have data to conduct such an assessment, the registrant is required to conduct a research program beginning by supplying to the Agency no later than December 1, 2001, a draft protocol which would be finalized using EPA comments on the protocol. The final protocol must be submitted to the Agency no later than March 15, 2002 and the research must commence in the 2002 growing season. Research topics must include, but are not be 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, determine whether insect pest emergence is in synchrony with pests emerging from *Bt* cotton, the proximity of alternate hosts to *Bt* cotton, and refine or construct 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.

To study whether *Bt*-resistant cotton bollworm would survive supplemental insecticidal treatments and increase the potential effectiveness of non-*Bt* refuges, research studies must be conducted 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, the registrant 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.

These study protocols, the interim reports, and/or the final reports cannot be claimed as CBI unless a substantiation of the CBI claim is made at the time the protocols are submitted to EPA.

B. *Bt* Cotton Terms and Conditions of the Amended Registration

1. Expiration Date

The subject registration will automatically expire on midnight September 30, 2006 except for the external, unsprayed refuge option which will expire September 30, 2004. EPA intends to review the data specified in the data requirements concerning alternate hosts and chemical insecticide sprays on *Bt* cotton, and decide in 2004 whether the new data support continuation of an external, unsprayed refuge as part of a larger requirement that would also likely involve alternative host plants. If these data support the continued availability of the external, unsprayed refuge option, EPA may approve an amendment to this registration to maintain the availability of this option.

2. Required Data

The protocols and data described in section III. A. above must be submitted to the Agency as part of the terms and conditions of the amendment to the Cry1Ac protein product registration. The following table outlines the protocols and data as well as the due dates for these.

Data	Description	Due Date
Residue Analytical Methods	Method submitted, but independent laboratory validation required	June 1, 2002
Protein Expression	Expression data provided for initial registration; confirmatory data required to provide consistency across <i>Bt</i> crops	March 15, 2003
Amino Acid and DNA Sequence	Stepwise 8 amino acid analysis	March 15, 2003
Cry Protein Levels in Soil	Supplemental studies; protocol to be submitted before studies are initiated	Protocol March 15, 2002; interim report January 31, 2003; final report January 31, 2004
Non-target Insects	Either existing studies or protocol and studies	Existing studies or protocol March 15, 2002; studies due January 31, 2005
IRM–north/south movement of cotton bollworm	Potential for north to south movement of cotton bollworm	January 31, 2004
IRM–Alternate Hosts/Insecticide sprays	Alternate host data as an effective refuge and sprays with chemical insecticides to enhance <i>Bt</i> cotton IRM effectiveness	Protocol December 1, 2001; final protocol March 15, 2002; interim report March 15, 2003 and final March 15, 2004

3. Gene Flow Containment Provisions

As discussed in the data requirements section above, the October 2000 SAP meeting supported EPA’s regulatory decisions to prohibit commercial cotton production in southern Florida and Hawaii where wild (or feral) cotton plants are known to exist. The SAP strongly supported EPA’s risk assessment. The most obvious concern is the development of weediness, but also concerns of biodiversity and loss of genes that might provide value in plant breeding have been considered.

Adequate data do not exist to complete a full risk assessment on the effects of the *Bt* Cry1Ac protein in wild cotton. Until thorough research on the impacts of gene flow can be completed, restriction on where *Bt* cotton can be planted are being implemented.

In light of the lack of basic biological data (*e.g.*, pollinator ecology, compatibility/sterility factors, potential impact of *Bt* on herbivores, distribution of native populations) on *G. tomentosum*, the wild Hawaiian cotton, conservative measures are needed to mitigate hybridization with cultivated cotton on these islands. Similarly, the paucity of data on the distribution of feral cotton in the U.S. Virgin Islands and Puerto Rico indicates the following terms and conditions must be instituted to mitigate gene flow concerns:

- a. No planting of *Bt*-cotton south of Route 60 (near Tampa) in Florida,
- b. Commercial culture of *Bt*-cotton is prohibited in the state of Hawaii,
- c. Test plots or breeding nurseries established in Hawaii must be surrounded by 24 border rows of a suitable pollinator trap crop regardless of the plot size and must not be planted within 3 miles of *Gossypium tomentosum*,
- d. Commercial culture, experimental plots and breeding nurseries of *Bt*-cotton are prohibited in the U.S. Virgin Islands, and
- e. Commercial culture of Bollgard™ cotton is prohibited in Puerto Rico. Test plots or breeding nurseries established on the island of Puerto Rico must be surrounded by 24 border rows of a suitable pollinator trap crop regardless of the plot size and must not be planted within 3 miles of feral cotton plants.

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.

4. Insect Resistance Management (IRM) Program

The Agency has determined that the unrestricted use of Cry1Ac in cotton is likely to lead to the emergence of resistance in one or more of the target insect pests unless measures are used to delay or halt the development of resistant insects. Because some cotton pests also attack other crops, not only would the emergence of resistance affect the benefits of Cry1Ac cotton, such insect resistance could also affect the efficacy of *Bt* corn products and microbial formulations of *Bt*. The loss of *Bt* as an effective pest management tool – in cotton or other crops – could potentially have serious adverse consequences for the environment to the extent that growers would shift to the use of more toxic pesticides and a valuable tool for organic farmers would be lost. The emergence of resistance in cotton pests could also have significant economic consequences for cotton growers. Therefore, EPA is requiring the registrant to implement an Insect Resistance Management (IRM) program to mitigate the possibility that pest resistance will occur.

The required IRM program for *Bt* cotton has 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 the registrant to prepare and require *Bt* cotton users to sign “grower agreements” which impose binding contractual obligations on the grower to comply with the refuge requirements;
- 3] Requirements for the registrant to develop, implement, and report to EPA on programs to educate growers about IRM requirements;
- 4] Requirements for the registrant to develop, implement, and report to EPA on programs to evaluate and promote growers’ compliance with IRM requirements;
- 5] Requirements for the registrant 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 the registrant to develop, and if triggered, to implement a “remedial action plan” which would contain measures the registrant would take in the event that any insect resistance was detected as well as to report on activity under the plan to EPA;
- 7] Submit 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:

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, pheromones, 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. This option expires after the 2004 growing season unless extended by amendment as described below. EPA intends to review the data specified in the data requirements concerning alternate hosts and chemical insecticide sprays applied to *Bt* cotton, and decide in 2004 whether the new data support continuation of an external, unsprayed refuge as part of a larger requirement that would also likely involve alternative host plants. If these data support the continued availability of the external, unsprayed refuge option, EPA may approve an amendment to this registration to maintain the availability of this option.

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 pheromones 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 pheromones 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 2002 growing season. EPA will evaluate the community refuge program following the 2002 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. The registrant must implement the 2002 community refuge pilot program as described in the Bollgard® Cotton 2002 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 the registrant by May 31, 2002. The registrant 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 the registrant or EPA as part of the compliance program.

The registrant 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 first phone audit shall occur no later than June 30, 2002 and the second phone audit shall occur no later than November 30, 2002. 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 the registrant under section 3.c. below.

The registrant must provide a written report to EPA at the end of the 2002 growing season on community refuge use and compliance (due by January 31, 2003).

The registrant must conduct a review of the community refuge program and submit that review to the Agency as to any proposed changes by January 31, 2003. An appropriate amendment for any proposed changes must be submitted to the Agency.

At the request of the registrant and based on EPA's review of the results of the 2001 community refuge pilot program, the requirements for the 2002 pilot program may be modified.

Rationale for Refuge Requirements:

In deciding on the size, proximity, configuration, and care of the non-*Bt* cotton refuge, EPA has taken into account a number of factors. EPA has used models developed to predict the estimated time that resistance would develop to compare IRM strategies for *Bt* crops. Because these predictive models cannot be validated without actual field resistance, they have limitations and the information gained from the use of such models can only be used as a part of the weight of evidence determination conducted EPA to assess the risks of resistance developing in target pest populations. EPA agrees with the 2000 SAP that models are an important tool in determining appropriate *Bt* crop IRM strategies and that model design should be peer reviewed and parameters validated. In the absence of field resistance, EPA agrees with the 2000 SAP that models are “the only scientifically rigorous way to integrate all of the biological information available, and that without these models, the Agency would have little scientific basis for choosing among alternative resistance management options.” While the absolute number of years to resistance is not precisely determined from the models, the relative difference in effectiveness between refuge options can be determined. Thus, the utility of the models is not that they make accurate quantitative predictions, rather, it is that they enable the Agency to make informed judgments of the potential effects of using various refuge options.

EPA has used at least five models in its comparative evaluation of refuge options. Each of these models has limitations based on the assumptions in the models. For example, the predictions generated by the models are very sensitive to assumptions about the genetics of resistance (gene frequency and functional dominance) about which little, if anything, is known. Each of these models has provided the Agency useful comparisons of refuge options. The Agency recognizes that the predicted years are not absolute, but provide a measure of the relative likely success of various refuge options (in terms of predicted years to resistance). EPA recognizes, however, there is uncertainty in the predicted outcomes of these models. The predictive reliability of the models increases as other factors such as level of *Bt* crop adoption, level of grower IRM compliance, fitness costs of resistance to the insect, presence and availability of alternate insect host plants, spatial components, stochasticity, and pest population dynamics are included. Such parameters, however, serve to increase the reliability of the predicted model results only to the extent that the inputs are verifiably validated.¹

¹Certain models assume 100% compliance and 100% adoption of *Bt* cotton. Both of these assumptions are not realistic based on data evaluated by the Agency. However, in the absence of empirical information that would predict the impact of lower than 100% compliance or adoption on the likelihood resistance, the assumptions of 100% compliance and 100% adoption are both reasonable. One hundred percent adoption has not occurred, and therefore if models were able to address this factor, they might predict a somewhat longer time-to-resistance with less than 100% adoption. Likewise, a model may assume only that all growers fully comply with applicable refuge requirements. Although compliance with IRM requirements by

Given the uncertainty of predictive models, EPA has asked for additional data to evaluate whether other factors, such as, alternate hosts, level of compliance, and level of adoption alter the predictions of the models. Until such time as these additional data become available, some of the models predictions may be overly conservative. However, given that EPA considers the development of resistance to be a significant adverse effect, the Agency believes it is prudent to err on the side of conservative regulatory practice.

EPA believes that resistance is not occurring in the field based on the available resistance monitoring information. After five years of analyzing resistance monitoring data (1996-2000), there is no evidence of tobacco budworm, cotton bollworm, or pink bollworm resistance to the Cry1Ac delta-endotoxin produced by *Bt* cotton. At this time, the Agency believes that this empirical data substantiate the success of the external unsprayed, external sprayed, and embedded refuge options to delay resistance. In addition, the Agency is mandating additional improvements to the current resistance management programs that will improve the detection of resistance.

In addition, to the use of predictive models and resistance monitoring data, EPA also weighed practical considerations in deciding which refuge options to allow. Based upon the currently available scientific data and information, the external, sprayed refuge option and the embedded refuge option both appear to provide an adequate time-to-resistance for *Bt* cotton. Where those refuge options are employed, EPA thinks there is limited chance of insect resistance over the next

cotton growers is generally high, some growers do not fulfill every requirement; this difference would likely lead to a somewhat shorter time-to-resistance. However, without additional data, it is not known how the rates of compliance or adoption will impact model predictions positively or negatively, nor is it known what impact on the uncertainty of these models these assumptions have. With respect to incorporation of a parameter addressing alternate hosts, as discussed in the Agency's risk assessment document, two SAPs (1998 and 2000) stated explicitly that alternate hosts for tobacco budworm and cotton bollworm could not be used as a refuge until there were empirical data to support their inclusion. To be effective, alternative hosts must produce susceptible, reproductively active insects at the same time as the *Bt* cotton acreage is producing potentially resistant, reproductively active insects. In addition, the alternative host plants must be close enough for such susceptible insects to mate with the potentially resistant insects on the *Bt* cotton fields. Unfortunately, the available data are not sufficient on the biological equivalence of the insects produced on the various host plants to evaluate the timing issue. Similarly, EPA does not have adequate information on the size and proximity of such potential alternative host acreage to *Bt* cotton fields to evaluate how likely insects from the alternative hosts would be to mate with potentially resistant insects. Without adequate data to address these data deficiencies, there is no basis to rely upon alternate hosts to provide suitable numbers of susceptible TBW or CBW. However, if alternate hosts can be empirically validated to function as a refuge, the models may predict longer time-to-resistance for an IRM approach. Such information does not currently exist; therefore, only non-*Bt* cotton may be used as a refuge.

several years. In some parts of the country, however, neither the external sprayed nor the embedded refuge options appear to be economically feasible for *Bt* cotton growers because these areas have high level of resistant TBW. In these cases, any sprayed refuge (whether embedded or external) would mean additional yield losses caused by the ineffective alternatives because of TBW resistance. Unless the external, unsprayed option is allowed, EPA would expect many growers to either stop growing cotton or shift to sprayed refuge with limited pesticide efficacy. Some growers would likely plant non-*Bt* cotton and spray it with conventional insecticides, some of which may be less efficacious, more costly, and more hazardous to human health and the environment. *Bt* cotton has been shown to replace insecticides with high toxicity to birds, fish, and people. This would mean a likely increase in overall insecticide use to control lepidopteran cotton pests. Shifts to conventional insecticide use either on sprayed or embedded refuges would likely lead to a reduction in overall grower and environmental benefits. Therefore, EPA believes it is preferable from an environmental and public health perspective for such growers to use the *Bt* cotton and thus, has decided to retain the external, unsprayed option for a time period that is less than the full duration of this time-limited registration, while additional data are being gathered to further characterize the actual risk of resistance development that use of this refuge option poses.²

EPA believes that it is imprudent to allow the external, unsprayed refuge option, described above, for more than a limited period of time, because current data indicates that this option has a significantly greater likelihood of insect resistance than either of the other refuge options. The 2000 SAP stated that the external, unsprayed option poses the highest risk to resistance evolution especially for cotton bollworm. Therefore, the external, unsprayed option expires after three growing seasons (September 30, 2004). During the next two years, the registrant is required to develop considerable new data on alternative host plants as possible effective refuges. In addition, the registrant is required to submit protocols by December 1, 2001, to begin field tests on alternative hosts and chemical insecticide sprays on *Bt* cotton, and to provide annual reports each January 31st. If any of these terms and conditions are not met, the external, unsprayed refuge option will be eliminated. If, based upon these, and any other pertinent data, the registrant requests an amendment to the registration extending the expiration date of the external, unsprayed option, EPA will conduct a comprehensive assessment of whether all relevant data support such regulatory action, as part of a larger requirement that would also likely involve alternative host plants. The data requirements regarding alternative host plants are described in section A.7.b. above.

b. Grower Agreements

²EPA considered other possibilities for 95:5 external, unsprayed refuge, e.g. a 90:10 external, unsprayed option. Upon review of the public comments, EPA concludes that increasing the size of the external unsprayed refuge would result in unacceptable economic losses and/or higher conventional insecticides use for those growers selecting the external, sprayed or embedded refuge options.

In addition to describing the standards for an effective refuge program as part of the Cry1Ac cotton IRM program, EPA believes it is important that there be a system to ensure a high level of compliance with such standards. The first element of such a system is a mechanism to create a legally enforceable obligation on *Bt* cotton growers to comply with the refuge program. This is accomplished through “grower agreements.” While the registrant 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] The registrant 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, 2002.

4] The registrant must continue to use its current grower agreement, and submit to EPA by November 1, 2001 a copy of that agreement. If the registrant 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, the registrant must submit to EPA the text of such changes to ensure that it is consistent with the terms and conditions of the amendment.

5] The registrant must establish a system which is reasonably likely to assure that persons purchasing the *Bt* cotton sign grower agreement(s), and must provide by December 1, 2001 a written description of that system.

6] The registrant 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, 2003 and annually thereafter, the registrant shall provide EPA with a report on the number of units of the *Bt* cotton seed shipped and not returned and the number of such units that were sold to persons who have signed grower agreements. The report shall cover the time frame of the twelve-month period covering the prior October through September. Note: the first report shall contain the specified information for the time frame starting with the date of registration and ending September 30, 2002.

8] The registrant 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.

EPA believes that this set of requirements collectively will enable the Agency to determine whether the registrant is satisfying the fundamental condition on its registration that growers, who purchase the *Bt* cotton product sign a legally enforceable grower agreement which imposes on them a legal obligation to comply with the current IRM program.

c. IRM Education and IRM Compliance Monitoring Programs

Ensuring compliance with the IRM program involves both educating growers about their obligations and monitoring the extent to which growers comply. The *Bt* cotton product registration contains conditions designed to make sure that the registrant carries out effective IRM education and compliance monitoring programs, specifically:

- 1] The registrant 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 will be provided to EPA for their records. The program shall involve at least one written communication annually to each Bollgard cotton grower separate from the grower agreement. The registrant shall coordinate its education program with educational efforts of other organizations, such as the National Cotton Council and state extension programs.
- 2] Annually, the registrant 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, 2002 and annually thereafter, the registrant 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] The registrant 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. The registrant shall

prepare and submit by January 31, 2002 a written description of its compliance assurance program. Other required features of the program are described in paragraphs 5] - 11] below.

5] The registrant shall establish and publicize a “phased compliance approach,” i.e., a guidance document that indicates how the registrant 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. The registrant shall provide a written summary of the results of the prior year’s survey to EPA by January 31 of each year. The registrant shall confer with EPA on the design and content of the survey prior to its implementation.

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

8] The registrant 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, the registrant shall take appropriate action, consistent with its “phased compliance approach,” to promote compliance.

9] The registrant 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, the registrant shall take appropriate action, consistent with its “phased compliance approach.”

10] If a grower, who purchases *Bt* cotton for planting, was specifically identified as not being in compliance during the previous year, the registrant 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, 2002 and annually thereafter, the registrant 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 the registrant, the number of tips investigated, the percent of growers who were not complying with the IRM requirements, and the follow-up actions taken.

12] The registrant 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 expressed in cotton is conditioned on the registrant carrying out appropriate programs to detect the emergence of insect resistance as early as possible. The goal of resistance monitoring is to detect resistance at a low enough resistance allele frequency so that changes to the insect resistance management plan can be made to increase the longevity of the product and prevent field failure. 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] The registrant will develop and ensure the implementation of a plan for resistance monitoring for *Heliothis virescens* (tobacco budworm) and *Helicoverpa zea* (cotton bollworm). The plan shall include provision for conducting annual studies to evaluate any potential change in susceptibility of tobacco budworm and cotton bollworm population to Cry1Ac protein. 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 tobacco budworm and cotton bollworm are important pests with a goal of having sites in AL, LA, AR, MS, FL, VA GA, NC, SC, TN, and TX.

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

3] The registrant shall provide a description to EPA of its resistance monitoring plan by January 31, 2002. The description shall include: sampling (number of locations and samples per locations), sampling methodology, bioassay methodology, standardization procedures, detection

technique and sensitivity, and the statistical analysis of the probability of detecting resistance.

4] The registrant 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 caterpillar, cotton leafperforator and European corn borer. The registrant 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. The registrant will investigate all damage reports. See Remedial Action Plans section below.

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

e. Remedial Action Plans

Specific remedial action plans are required for *Bt* cotton for the purpose of containing resistance and perhaps eliminating resistance if it develops. There are two types of situations, first suspected resistance and second confirmed resistance.

1) Suspected Resistance

EPA defines “suspected” resistance to mean, in the case of reported product failure, that:

- the cotton in question has been confirmed to be *Bt* cotton
- the seed used had the proper percentage of cotton expressing *Bt* protein;
- the relevant plant tissues are expressing the expected level of *Bt* protein; and
- it has been ruled out that species not susceptible to the protein could be responsible for the damage, that no climatic or cultural reasons could be responsible for the damage, and that other reasonable causes for the observed product failure have been ruled out.

The Agency does not interpret “suspected resistance” to mean grower reports of possible control failures, nor does the Agency intend that extensive field studies and testing to fully scientifically confirm insect resistance be completed before responsive measures are undertaken.

If resistance is “suspected,” the registrant must instruct growers to do the following:

- Use alternate control measures to control the pest suspected of resistance to *Bt* cotton in the affected region.
- Destroy crop residues in the affected region immediately after harvest (i.e. within one month) with a technique appropriate for local production practices to minimize the

possibility of resistant insects overwintering and contributing to the next season's pest population.

2) Confirmed Resistance

The registrant assumes responsibility for the implementation of resistance mitigation actions undertaken in response to the occurrence of resistance during the growing season. When resistance has been confirmed, the registrant must stop sale immediately and distribution of *Bt* cotton in the remedial action zone (may be less than a single county, single county, or multiple counties) where the resistance has been shown until an effective local mitigation plan approved by EPA has been implemented.

3) Remedial Action Plan for Pink Bollworm

The Arizona *Bt* Cotton Working Group has produced "A Remedial Action Plan for Pink Bollworm Resistance to *Bt* Cotton in Arizona" which is thorough and EPA believes is very workable. In addition, the October 2000 SAP agreed that this was a good model Remedial Action Plan. If resistance involves the pink bollworm (*Pectinophora gossypiella*), the registrant must implement the Arizona *Bt* Cotton Working Group's Remedial Action Plan. The registrant must obtain approval from EPA before modifying the Arizona *Bt* Cotton Working Group's Remedial Action Strategy. The Arizona *Bt* Cotton Working Group's Remedial Action Plan can be found in Appendix 1.

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

The October 2000 SAP stated that was no remedial action plan in place for *Helicoverpa zea* (cotton bollworm) and *Heliothis virescens* (tobacco budworm), but that Arizona plan could provide a useful model. Based upon the Arizona model, an interim Remedial Action Plan for cotton bollworm and tobacco budworm has been develop and must be implemented by the registrant if suspected or confirmed resistance is found. The Interim Remedial Action Plan for Cotton Bollworm and Tobacco Budworm is contained in Appendix 2. After consultation with cotton growers and academic experts, the registrant plans to submit a revised Remedial Action Plan by May 15, 2002 for EPA's review and approval. The registrant must obtain approval from EPA before modifying the Remedial Action Plan for Cotton Bollworm and Tobacco Budworm.

5. Annual Reports

The registrant will provide an annual report to EPA on its Cry1Ac PIP 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 III. A. 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.

IV. Regulatory Position on *Bt* Cotton

The *Bt* cotton product was registered for commercial use in October 1995 as a conditional registration under FIFRA Section 3(c)(7)(B). The data reviewed for the initial registration as well as new data and reports received, results of public meeting, hearings, workshops, forums, and Scientific Advisory Panel meetings, and public comments received regarding the *Bt* crops reassessment have been taken into consideration. The scientific assessment has included product characterization, human health effects, gene flow, effects on non-target organisms, ecological exposure, insect resistance management, and benefits. Over the last six years, new data and information have been provided to the Agency in each of these areas and these data have been incorporated into the science assessment and has been taken into account in making regulatory decisions.

For example, new mechanisms to analyze the DNA sequences inserted into the plant have been developed. The genetic material inserted into cotton has been analyzed using new methods such as genome walking, PCR, and cosmid libraries. These data and the data already available on the transformation system used, characterization of the DNA inserted into the plant, inheritance and stability of the DNA after transformation, and protein characterization and expression support the amendment to extend the registration for Cry1Ac protein expressed in cotton.

Tests have shown no toxicity to mammals from the Cry1Ac protein; the protein is readily digestible in gastric fluids and are non-glycosylated, the protein is inactivated by typical food processing, and anticipated exposure to the protein from farm workers are negligible. The Cry1Ac protein acute oral toxicity data submitted demonstrated no effects at the relatively high dose level of 5,000 mg/kg. Cry1Ac protein is degraded between two minutes and seven minutes by gastric fluid *in vitro*. Exposure via the skin or inhalation is not likely since the Cry1Ac protein is contained within cotton plant cells which essentially eliminates or reduces exposure routes to negligible. Oral exposure, at very low levels, may occur from ingestion of processed products and drinking water. Worker exposure to the Cry protein via seed dust is also expected to be negligible because of the low amount of protein expressed in seeds of the transformed plants. Taken in total, these data allow the Agency to make a determination that for human health, there is a reasonable certainty that no harm will result from aggregate exposure to the U.S. population, including infants and children, to the Cry1Ac protein and the genetic material necessary for its production. Thus, EPA concludes that there are no adverse effects on human health from the use of Cry1Ac protein expressed in cotton.

EPA has also reviewed the original data base and the new data, information, and comments regarding ecological effects. EPA has reviewed the potential for gene capture and expression of the Cry1Ac endotoxin in cotton by wild or weedy relatives of cotton in the United States, its possessions or territories. EPA has concluded that there is a possibility for gene transfer in limited geographic locations where wild or feral cotton relatives exist. This transfer is of concern because

1) traits which enhance the survival, invasiveness or adaptability of a plant have the potential to increase the frequency of that trait (allele) in the recipient population and result in a shift in community dynamics (*e.g.*, species abundance, distribution) for multiple species, 2) the native genome of any wild species is effectively altered by the introduction of an adaptive trait (*e.g.*, resistance to insects, diseases, stress) and a net loss in the biodiversity of the recipient species may occur as alleles or even biotypes of the species are lost through this genetic introduction and selection, and 3) wild or feral species which are genetically compatible with crop plants and other non-domesticated plant species, and are recipients of novel traits, may transfer these traits in a reciprocal fashion to these related species in subsequent generations. Therefore, EPA has imposed restrictions on the planting of commercial cotton in southern Florida, Hawaii, Puerto Rico, and the U.S. Virgin Islands. In addition, restrictions to prevent gene flow have been imposed for test plots and breeding nurseries in Hawaii and Puerto Rico although the registrant may provide data which will allow EPA to ease or remove these restrictions in the future.

The Agency has concluded that the weight of evidence indicates no unreasonable adverse effects of Cry1Ac protein expressed in cotton to non-target wildlife or beneficial invertebrates. EPA further believes that cultivation of Cry1Ac cotton may result in fewer adverse impacts to non-target organisms than result from the use of chemical pesticides. However, EPA is requiring insect census estimates from representative fields to determine if there are long-term adverse impacts from the use of *Bt* cotton and field tests of Cry1Ac protein accumulation and/or persistence in soil under a range of conditions typical of *Bt* crop cultivation as confirmatory data.

In the ecological effects testing done, no treatment related effects were observed in Bobwhite quail fed Cry1Ac cottonseed meal as part of their diet. No measurable deleterious effects from the Cry1Ac protein on honey bee larvae, honey bee adults, parasitic wasps, Ladybird beetles, green lacewings and Collembola (springtails) were observed in submitted studies. The larvae of endangered Lepidoptera species in cotton growing counties (Quino Checkerspot butterfly, Saint Francis' Satyr butterfly and Kern Primrose Sphinx moth) are not going to be exposed to Cry1Ac protein because their habitats do not overlap with cotton fields.

Limited data do not indicate that Cry proteins have any measurable effect on microbial populations in the soil. Horizontal transfer from transgenic plants to soil bacteria has not been demonstrated. Purified microbially produced Cry1Ac protein produced a DT₅₀ (Degradation Time) of 9.3-20.2 days. Ground, lyophilized Cry1A(c) cotton line 931 tissue produced a DT₅₀ of 41 days. Based upon estimates of 60,000 plants per acre, a total of 1.44 grams of Cry protein per acre would enter the soil when the cotton plants are incorporated after harvest.

The issue of insect resistance management has generated more data, meetings, and public comments than all of the other sections covered in this BRAD. Insect resistance management (IRM) is the set of practices aimed at reducing the potential for insect pests to become resistant to a pesticide. *Bt* IRM is of great importance because of the threat insect resistance poses to the

future use of *Bt* plant-protectants and *Bt* technology as a whole. EPA considers protection of insect (pest) susceptibility of *Bt* to be in the “public good.” EPA has determined that development of resistant insects would constitute an adverse environmental effect. In order to delay the development of insect resistance to *Bt* cotton by maintaining insect susceptibility, growers must choose at least one of structured refuge (a portion of the total acreage using non-*Bt* seed) options listed in Section III.B.4.a. above. In addition, the IRM program requires 1) anyone purchasing *Bt* cotton to sign a grower agreement which contractually binds the grower to comply with the IRM program and that there will be a mechanism by the year 2003 by which every grower affirms their contractual obligations to comply with the IRM program, 2) an IRM education program, 3) an IRM compliance monitoring program including a third party compliance survey and mechanisms to address non-compliance, 4) and insect resistance monitoring program for each target insect pest, 5) remedial action plans to be implemented if resistance does develop, and 6) annual reporting of the IRM (and other) activities. No other pesticide products than the *Bt* crop products have such extensive IRM requirements.

In addition to assessing the risks from the use of Cry1Ac expressed in cotton, EPA has evaluated the benefits from the use of this product. Direct grower benefits include reduced pesticide use, improved crop management effectiveness, reduced production costs, improved yield and profitability, reduction in farming risk, and improved opportunity to grow cotton in areas of severe pest infestation. Total monetary grower benefits from the use of *Bt* cotton are between \$60 million and \$126 million. Indirect benefits may include improved populations of beneficial insects and wildlife in cotton fields, reduced pesticides runoff, reduced air pollution and waste from the use of chemical insecticides, improved farm worker and neighbor safety, and reduction of fossil fuel use. EPA believes that cultivation of Cry1Ac cotton may result in fewer adverse impacts to non-target organisms than result from the use of chemical pesticides.

Pursuant to FIFRA Section 3(c)(7)(A), EPA may conditionally amend the registration of a pesticide if the Agency determines (i) that the pesticide and proposed use are identical or substantially similar to a currently registered pesticide and use thereof, or differs only in ways that would not significantly increase the risk of unreasonable adverse effects on the environment, and (ii) approving the amendment in the manner proposed by the applicant would not significantly increase the risk of unreasonable adverse effect on the environment. FIFRA defines “unreasonable adverse effects on the environment” in pertinent part as: “any unreasonable risk to man or the environment, taking into account the economic, social, and environmental costs and benefits of the use of any pesticide” Thus, the FIFRA unreasonable adverse effects standard requires EPA to balance the risks and benefits of using the pesticide in reaching its regulatory decision.

EPA finds that the use of Cry1Ac expressed in cotton will not significantly increase the risk of unreasonable adverse effects on the environment. This finding, however, applies only to the use of Cry 1Ac protein expressed cotton under the terms and conditions of registration specified below, and only for the limited time period of 5 additional years (to September 30, 2006), except for the

external, unsprayed refuge option which will expire September 30, 2004. The following sections set forth the basis for EPA's finding in general, and the basis for the decision to approve the registration subject to the specific terms and conditions identified below.

General Finding

EPA's finding that Cry1Ac protein expressed in cotton will not significantly increase the risk of unreasonable adverse effects on the environment is based on the analysis contained in the preceding sections of this BRAD and the specific terms and conditions that are imposed upon this registration, as set forth in Section III. In general terms, EPA concludes that use of Cry1Ac expressed in cotton is effective at controlling significant lepidopteran pests of cotton, including tobacco budworm, cotton bollworm, and pink bollworm. Therefore, this product has clear benefits for users. Beyond these economic benefits, EPA determines that Cry1Ac provides benefits as an alternative to the use of other cotton insecticides in that use of Cry1Ac protein expressed in cotton results in less human and environmental risk. In addition, EPA finds that the use of this product, subject to the specific terms and conditions set forth below, would not pose risks to human health or to non-target species. EPA also concludes that the use of Cry1Ac expressed in cotton raises concerns with respect to: 1) the risk of gene flow to feral cotton species; and 2) insect resistance management. As discussed below, the registration for Cry1Ac protein expressed in cotton is subject to specific terms and conditions that effectively restrict the use of the product in ways that EPA determines will adequately mitigate these concerns. Therefore, EPA determines that the allowed use will not significantly increase the risk of unreasonable adverse effects on the environment. Finally, EPA has identified the need for certain confirmatory data on potential accumulation of Cry1Ac protein in soil and field impacts of Cry1Ac protein on non-target species.

Insect Resistance Management (IRM) Program

EPA has determined, based on all available scientific data and information, that the unrestricted use of Cry1Ac expressed in cotton is likely to lead to the emergence of resistance in one or more of the target insect pests unless adequate and appropriate measures are used to delay or halt the development of resistant insects. Because some cotton pests also attack other crops, not only would the emergence of resistance adversely impact the efficacy of Cry1Ac protein expressed in cotton, such insect resistance would also likely adversely impact the efficacy of *Bt* corn products and microbial formulations of *Bt*. The loss of *Bt* as an effective pest management tool – in cotton or other crops – could potentially have serious adverse consequences for the environment to the extent that growers shifted to the use of more toxic pesticides and loss of a valuable tool for organic farmers. The emergence of resistance in cotton pests could also have significant economic consequences for cotton growers. Therefore, EPA is requiring the registrant to implement an Insect Resistance Management (IRM) program to mitigate the possibility that pest resistance will occur.

Appendix 1

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

I. Definitions

Definition #1. Putative Resistance Event--A Cautionary Alert

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

Definition #2. A Verified Resistance Event.

A putative resistance event becomes verified if three conditions are met:

A sample of 1000 bolls yields =3% containing large larvae (=3rd instar), pupae, or PBW exit holes.

An ELISA test for *Bt* toxin yields a positive response for *Bt* toxin in a sample of 25 young bolls collected from plants on which PBW larvae were found in the cotton field of interest.

Standardized laboratory bioassays demonstrate that the PBW population of interest is significantly less susceptible to Cry1A(c) toxin than were baseline populations in 1997 (Simmons et al. 1998 and unpublished).

II. Remedial Action

Putative Resistance Event: Year of First Detection.

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

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

survival of resistant pink bollworm. Destroy crop residue as indicated above.

Verified Resistance Event: Year of First Detection.

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

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

The '*Bt* remedial action zone' should be delineated using GPS mapping technology currently in use at the ACRPC. This will ensure accurate records of locations of verified resistance. The remedial action zone should include all sections of land falling within 6 miles of the perimeter of the section(s) of land in which verified resistance events occurred (see figure below).

At such time as fields with verified resistance are detected in >3 different townships within a particular cotton growing region, the entire region may be designated as a *Bt* resistance remedial action zone.

Verified Resistance Event: Next Year's Actions.

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

The ecological fitness of PBW resistant to Cry1Ac is not known at this time and the dynamics of resistance in the field will likely be influenced by factors including overwintering survival of resistant larvae, intensity of resistance to Cry1Ac, and growth and survival of resistant PBW on *Bt*

and non-*Bt* plants. Therefore, new information derived from field and laboratory studies currently underway will be pivotal for determining at what frequency of resistance to Cry1Ac could use of *Bt* cotton expressing Cry1Ac reasonably be resumed within an area previously designated as a *Bt* remedial action zone.

It is assumed that published University recommendations for monitoring and chemical control of pink bollworm will be followed within remedial action zones in order to limit survival of resistant pink bollworm. Additionally, timely crop termination (no top-crop) and early and

thorough crop destruction, as detailed above, is strongly encouraged. Releases of sterile pink bollworm and parasitic nematode treatments should also be considered.

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

III. Organizational Roles

The Arizona Department of Agriculture should serve a central role in implementing this plan, compiling statistics on use of *Bt* cotton, and promoting compliance with remedial action.

Consideration should be given on a case-by-case basis for making funds available to compensate producers for costs associated with implementing the remedial action measures recommended herein.

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

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

Appendix 2

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

1. Actions required for “suspected” resistance events (YEAR 1)

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

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

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

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

The registrant must instruct growers to use appropriate alternate control measures on the *Bt* cotton fields to control the potentially resistant pest populations in the subject field only.

The registrant will work with local consultants or state entomologists to monitor the subject field for the remainder of the season or until resistance is confirmed NOT to be the cause. The registrant will instruct growers to use alternate control measures based on the results of the discriminating dose bioassay indicating tolerance/resistance.

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

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

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

resistance from the field and in the surrounding general population.

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

Dose mortality, diagnostic/discriminating dose, allelic recovery, and other confirmatory tests (as described below) will be conducted with the collected larvae from fields in which resistant individuals may be present by the registrant and USDA/ARS/ and interested parties.

The following definition of resistance will be used for TBW and CBW:

A resistance event becomes verified if the progeny of the sampled TBW and/or CBW population exhibit the following characteristics in bioassays initiated with neonates:

- i) if there is > 5-10% survival and > 25% leaf area damaged in a 5-day bioassay using Cry1Ac-positive leaf tissue under controlled laboratory conditions. Note: Since there is not a high dose for CBW, this assay only applies to TBW
- ii) if standardized laboratory bioassays using diagnostic doses for TBW and CBW (as currently used by USDA/ARS/) demonstrate resistance has a genetic basis and confirmed survivorship in excess of 1% in a random population sample.
- iii) if an LC_{50} in a standard Cry1Ac diet bioassay exceeds the upper limit of the 95% confidence interval of the standard unselected laboratory population LC_{50} for susceptible CBW and TBW populations, as established by the ongoing baseline monitoring program.

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

Once resistance is confirmed, the registrant must notify EPA within 30 days and work with the Agency to establish a resistance mitigation plan.

Concurrently, surrounding *Bt* cotton fields will be monitored by the registrant and cotton consultants/state entomologists throughout the season for unusually high survival incidences of bollworm or tobacco budworm. Populations with unusually high survival will be collected and tested in the discriminating dose assay.

The registrant will instruct growers to arrange for early harvest of the crop and fall or spring plowing to reduce the potential for overwintering pupae.

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

subsequent year, the level of resistance, and the prevalence of the resistance.

3. Mitigation strategies required for YEAR 2 and beyond

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

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

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

The registrant will increase resistance monitoring in all affected areas and adjacent counties or other areas in which resistance may be likely.

More specific recommendations may be developed through winter meetings by the registrant and local cotton experts that are tailored to the specific pest/situation.

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

- a. Alternate control measures
- b. Reduce/eliminate *Bt* cotton use
- c. Reduce/eliminate cotton use
- d. Modify refuge requirements
- e. Lab generated susceptible male release (sterile if appropriate)
- f. Spring/early summer trap crops with alternate control measures

- g. Area wide virus treatments over spring hosts
- h. Continue field and resistance monitoring in expanded areas
- i. Use feeding or mating attractants in *Bt* fields to improve susceptible male (or female if using feeding attractants) movement and random-mating with resistant insects

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

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