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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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OFFICE OF PREVENTION,
PESTICIDES AND TOXIC
SUBSTANCES

OPP PUBLIC DOCKET

OCT 31 1995

MEMORANDUM

SUBJECT: Consideration of Section 3(c)(7)(B) Conditional Amendments for Monsanto Company's Plant-Pesticide *Bacillus thuringiensis* CryIA(c) δ-Endotoxin and the Genetic Material Necessary for Its Production in Cotton.

-DECISION MEMORANDUM-

FROM: Janet L. Andersen, Acting Director
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TO: Daniel M. Barolo, Director
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I. ISSUE

Should the Agency conditionally amend the existing FIFRA §3(c)(5) registration for limited plant propagation use to permit an additional use of the product in cotton pursuant to FIFRA §3(c)(7)(B)? The active ingredient in this pesticide product is the *Bacillus thuringiensis* CryIA(c) δ-endotoxin and the genetic material necessary for its production in cotton. "Genetic material necessary for its production" means the genetic material which comprise (1) genetic material encoding the CryIA(c) δ-endotoxin and (2) its regulatory regions. "Regulatory regions" are the genetic material that control the expression of the genetic material encoding the CryIA(c) δ-endotoxin, such as promoters, terminators, and enhancers. The limitations currently placed upon the use of the product include but are not limited to the acreage which may be planted, the duration of the registration, geographic areas where the product may be used, and post-harvest agricultural practices.

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II. SUMMARY AND REGULATORY BACKGROUND

Monsanto Corporation has submitted an application to amend the seed increase/hybrid production registration to expand this registration to full commercial use in cotton. A tolerance for this food use has been previously issued (Petition 4F4331, issued September 15, 1995). There is also an inert marker gene in this product (NPTII). The NPTII marker gene tolerance was issued on September 28, 1994 (3F4273).

The Agency published its proposed position on the regulation of pesticidal substances produced in plants (**59 FR 60496, November 23, 1994**). In the proposal, the Agency would designate the pesticidal substances produced by plants as plant-pesticides. In addition, the Agency issued proposed regulations that define certain categories of plant-pesticides that would be exempt from regulation under FIFRA and FFDCA. Plant-pesticides not exempt would be subject to regulation. The *Bacillus thuringiensis* δ-endotoxins are examples of plant-pesticides that would be regulated under the proposal.

On March 21, 1995, the Agency issued a limited registration that allows Monsanto Corporation to produce seed for seed increase and hybrid production, but that is limited in scope and duration.

The Biopesticides and Pollution Prevention Division (BPPD), Biological and Economic Analysis Division (BEAD), and the Pesticide Resistance Management Workgroup (PRMW) have evaluated the data submitted by Monsanto. Based on these data and other relevant information, BPPD believes that 1) the product will perform its intended function 2) the applicant has submitted/cited satisfactory data pertaining to the proposed additional use and 3) amending the registration by removing the acreage and crop disposition limitations contained in the original registration and adding food and feed uses would not significantly increase the risks of any unreasonable adverse effects to humans, nontarget organisms, or the environment. BPPD scientists have reviewed the information submitted with respect to health effects, and these data show that the product will be digested like any other protein and genetic material and will have no significant effects on human health. Likewise, the data submitted for ecological effects have identified no significant hazards to non-target organisms. The benefits data have been reviewed and the product has been found to be effective and the benefits of its use persuasive. The PRMW has reviewed the pesticide resistance management plans and has identified certain terms and conditions necessary to reduce the potential for development of insect resistance to *Bacillus thuringiensis* (Bt).

The amended registration for food and feed use of this product in cotton, if granted, would be conditional under section 3(c)(7)(B) of FIFRA. The data required all relate to the mitigation of resistance and resistance monitoring and are discussed in detail in section IV E. 2) of this memorandum. The conditionally required data were not listed as a requirement for this active ingredient prior to the date of the submission by Monsanto.

The Agency is also imposing terms and conditions of use as outlined in section III of this document to address resistance management concerns. These conditions and terms are being imposed to address concerns that insects will develop resistance to *Bt* plant-pesticides and *Bt* sprays which can be used on corn, cotton, and a variety of vegetable crops.

Monsanto has agreed to all of these conditions in writing and has submitted a revised label and grower guide incorporating these limitations and conditions.

III. PUBLIC COMMENTS

Thirty-eight individuals or organizations provided written comment for the proposed registration. All comments were positive in nature, noting the potential benefits of the plant-pesticide. The Agency benefits review is in general agreement with these comments.

Several commentors have expressed concerns (relative to the original application and a Science Advisory Panel meeting on March 1, 1995) regarding emergence of resistance to the active ingredient by the target pest. The Agency agrees with commentors that without a resistance management strategy, cross-resistance and/or multiple resistance could develop to the CryIA(c) δ-endotoxin such that there could be some deleterious impact on *Bt* microbial pesticides.

The Agency recognizes the risks associated with the development of resistance to the CryIA(c) δ-endotoxin and the need for a long-term implementable resistance management strategy. A review of the reports published by the USDA National Agricultural Statistics Service (Agrichemical Usage Vegetables, Field Crops) indicates that significant *Bt* usage occurs not only for cotton, but also for vegetable crops such as broccoli, cabbage, celery, eggplant, lettuce, bell peppers, spinach, and tomatoes. Chemicals registered for the target pests of cotton which are also registered for these vegetables include synthetic pyrethroid (fenvalerate, permethrin, fluvalinate, bifenthrin and cyfluthrin) organophosphate (chlorpyrifos, monitor, guthion) and carbamate (methomyl) pesticides which are potentially more hazardous to man and the environment than foliar *Bt* sprays.

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Therefore, a risk exists that resistance may not only occur for pests of cotton, but that those cotton pests which also feed on vegetable crops could spread this resistance into those agricultural systems as well, jeopardizing the utility of safer *Bt* products used on these crops.

Monsanto corporation has provided a resistance management plan in their submissions. It appears that Monsanto is committed to implementing a resistance management plan. OPP's Pesticide Resistance Management Workgroup's (PRMW) detailed technical analysis of the Monsanto resistance management strategy is discussed in the support document entitled:

"PRMW Technical Review of the Resistance Management Plan Proposed by Monsanto Company for the Bacillus thuringiensis CryIA(c) Delta Endotoxin Produced in Bollgard Cotton" September 27, 1995.

The Agency has made several recommendations for further research and actions to delay the development of resistance to CryIA(c) (discussed below in the recommendations section).

IV. SCIENCE ASSESSMENT

BPPD and BEAD scientists have reviewed the studies submitted by Monsanto along with other relevant information. These data and information indicate that the registration and use of the proposed product will have no adverse effects on human health, based upon extensive characterization studies, a mouse acute toxicity study, an in-vitro digestibility study, and the known properties of proteins and genetic material. The expression of the protein within the plant has been adequately described, and will result in only low levels of environmental exposure. Studies on non-target organisms indicate no concerns. There is a possibility for gene transfer in locations within the United States where wild or feral cotton relatives exist (Hawaii and Florida). Therefore BPPD is proposing containment provisions for these states. EPA has made a "no-effect" finding for endangered species.

As indicated in Section III. above, resistance management is a major concern for this product. The PRMW review of the Monsanto resistance management identified certain additional data requirements and limitations to the use of the proposed product. See section IV E. for a detailed discussion of these requirements and limitations.

A. PRODUCT CHARACTERIZATION

Monsanto submitted information which adequately described the CryIA(c) delta-endotoxin from *B.t.*, as expressed in cotton, along with the genetic material necessary for its production.

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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 CryIA(c) delta-endotoxin is sufficiently similar to that expressed in the plant to be used for mammalian toxicological purposes. Plant and microbially produced CryIA(c) 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.

1. **Product Characterization #1a:** Southern blot analysis on restriction digests of DNA extract from cotton line 531 and the parental Coker 312 showed that there is probably only one insert of the CryIA(c) gene cassette presented in the transformed line. The introduced gene appears to be genetically stable in the cotton according to the results of the progeny selfing and backcrosses with the elite lines. The amino acid sequence is homologous to the cryIA(b) gene from HD-1 for positions 1-466 and homologous to cryIA(c) for positions 467-1178 with a single exception of a leucine-serine 766 in the crystal portion of the protein cleaved prior to toxin activation. Western blot analysis of purified toxin, leaf tissue from cotton line 531 and the parental Coker 312 shows that trypsinized extracts have comigrating bands similar to that found in **B.t.k.** HD-73 protein reference material and commercial preparations.

2. **Product Characterization #2b:** B.t.k. HD-73 toxin isolated from either cotton line 531 or 931 were compared to the toxin expressed in **Escherichia coli (E. coli)** by SDS-PAGE, western blot, glycosylation and bioactivity. The data presented suggests the bacterially produced protein and that found in cotton are equivalent and suggests the bacterially produced **B.t.k.** HD-73 toxin can serve as a surrogate test substance in the toxicological tests to support the registration of transgenic cotton. The original data package for this study did not have a section describing the purification method to obtain the plant standard, and was classified as supplementary on that basis. Additional information on the purification method as described in "Assessment of Equivalence Between E. coli-produced and Cotton-produced Btk HD-73 Protein..." MRID 43152-02 were provided. The additional information was sufficient to clarify the extraction procedure and the study is now acceptable.

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3. **Product Characterization # 3c:** The delta-endotoxin from B.t.k. HD-73 (lot # 5025385) produced in E. coli containing plasmid (pMON10569) was purified, lyophilized and found to have the following characteristics: 4.5% moisture, 75.6% protein (amino acid analysis), 70% protein (BCA), 88% HD-73 specific protein (ELISA), 80% HD-73 specific protein (coomassie blue PAGE), 1.6 ug gram negative endotoxin/mg and no significant trace metals except for sodium, potassium and phosphate. The molecular weight of the B.t.k. HD-73 toxin was estimated to be 134.8 kD for the full length species and 77.1 kD for the tryptic fragment. The functional activity was found to be an LC50 of 0.28 ppm against Heliothis virescens.

4. **Product Characterization # 4d:** Ten insect pest species from 5 families were tested for their sensitivity to B.t.k. HD-73 protein. Only in the lepidopteran species was there significant mortality. In one study, the green peach aphid showed marginal effects from treatment with a tryptic digest of the CryIA(c) toxin from B.t.k. HD-73 which was not reproducible in a repeat test. The tryptic digest preparation positive control from a B.t.k species also showed higher mortality in the tobacco budworm test than that produced in E. coli.

B. HUMAN HEALTH

1. **Acute Oral Toxicity:** Ten male and female CD-1 mice per dose level were exposed by oral gavage to 500, 1000 and 4200 mg/kg bodyweight of E. coli produced B.t.k. HD-73 toxin. The controls were given the protein equivalent of 6340 mg/kg of bovine serum albumin. No mortalities or treatment related adverse effects were seen in either the treated or control mice. There were no observable dose related effects seen upon necropsy. CLASSIFICATION: Acceptable. Tox. Category IV.

2. **In Vitro Digestibility:** The *B.t.k.* HD-73 protein was rapidly degraded to fragments not recognized in a western blot after 7 minutes incubation in simulated gastric fluid (SGF) and was not active in a tobacco budworm (TBW) bioassay after SGF incubation. The in vitro digestibility assay provides useful information to predict the metabolic fate of the CryIA(c) protein and its potential as a food allergen. However, it is not clear how this protein assay's results relate to protein toxicity. Therefore the Agency also requested that an acute oral toxicity study be done to confirm the expected lack of toxicity indicated by the *in vitro* digestibility results.
CLASSIFICATION: Acceptable.

TOXICOLOGY ASSESSMENT

The toxicology data provided are sufficient to demonstrate that there are no foreseeable human health hazards likely to arise from the use of *Bacillus thuringiensis* CryIA(c) δ-endotoxin and the genetic material necessary for its production when used as a plant-pesticide in any cotton plant.

The submitted information along with an acceptable review of the data on pesticide expression in the plant is an adequate characterization of the B.t.k protein (CryIA(c) delta-endotoxin) as expressed in cotton. These data also support the use of the E. coli produced protein for the acute oral and in vitro digestibility studies.

The data submitted regarding potential health effects include information on the characterization of the expressed CryIA(c) δ-endotoxin in cotton, the acute oral toxicity, and *in vitro* digestibility of the δ-endotoxin.

Toxicity -

The Agency expects that proteins with no significant amino acid homology to known mammalian protein toxins and which are readily inactivated by heat or mild acidic conditions would also be readily degraded in an *in vitro* digestibility assay and have little likelihood for displaying oral toxicity.

The data submitted support the prediction that the CryIA(c) protein would be non-toxic to humans. When proteins are toxic, they are known to act via acute mechanisms and at very low dose levels [Sjobald, Roy D., et al. "Toxicological Considerations for Protein Components of Biological Pesticide Products," Regulatory Toxicology and Pharmacology 15, 3-9 (1992)]. Therefore, since no significant acute effects were observed, even at relatively high dose levels, the CryIA(c) δ-endotoxin is not considered acutely or chronically toxic. Adequate information was submitted to show that the test material derived from microbial cultures were biochemically and insecticidally similar to the δ-endotoxin as produced by the plant-pesticide in cotton. Microbially produced CryIA(c) δ-endotoxin was chosen in order to obtain sufficient material for mammalian testing. In addition, the *in vitro* digestibility studies indicate the δ-endotoxin would be rapidly degraded following ingestion.

The majority of proteins expressed in plants as plant-pesticides are not expected to present a risk of dermal or inhalation toxicity for two reasons. First, the expression level of the introduced protein is generally extremely low and the protein should be found internally in the plant, inside the plant cell wall, with little or no potential for direct dermal or inhalation exposure. Second, proteins found to be non-toxic by the oral route are not expected to be toxic by the pulmonary or dermal route of exposure. If the risk equation is considered (risk = hazard x exposure), the low to nil exposure to the protein by the dermal or inhalation route coupled with no demonstrated oral toxicity of the protein is consistent with a conclusion of insignificant risk by the dermal or inhalation route.

The genetic material necessary for the production of the *Bacillus thuringiensis* CryIA(c) δ-endotoxin are the nucleic acids (DNA) which comprise (1) genetic material encoding the CryIA(c) δ-endotoxin and (2) its regulatory regions. "Regulatory regions" are the genetic material that control the expression of the genetic material encoding the CryIA(c) δ-endotoxin, such as promoters, terminators, and enhancers. DNA is common to all forms of plant and animal life and the Agency knows of no instance where these nucleic acids have been associated with toxic effects related to their consumption. These ubiquitous nucleic acids as they appear in the subject active ingredient have been adequately characterized by the applicant. Therefore no mammalian toxicity is anticipated from dietary exposure to the genetic material necessary for the production of the *Bacillus thuringiensis* CryIA(c) δ-endotoxin in cotton.

Allergenicity -

Current scientific knowledge suggests that common food allergens tend to be resistant to degradation by heat, acid, and proteases, are glycosylated and present at high concentrations in the food. Submitted data indicate that the CryIA(c) δ-endotoxin is rapidly degraded by gastric fluid *in vitro*, is not present as a major component of food (i.e., is not found in cotton seed) and is apparently non-glycosylated or otherwise post-translationally modified when produced in plants.

Studies submitted to EPA done in laboratory animals also have not indicated any potential for allergic reactions to B. thuringiensis or its components, including the δ-endotoxin in the crystal protein. Recent *in vitro* studies utilizing simulated gastric fluid also confirm that the δ-endotoxin would be readily digestible *in vivo*, unlike known food allergens that are resistant to degradation.

Despite decades of widespread use of *Bacillus thuringiensis* as a pesticide (it has been registered since 1961), there have been no confirmed reports of immediate or delayed allergic reactions to the δ-endotoxin itself through oral, dermal and/or inhalation exposure to the microbial product. Several reports under FIFRA § 6(a)2 have been made for various *Bacillus thuringiensis* products with allergic reactions being reported. However, these reactions were determined not to be due to *Bacillus thuringiensis* itself or any of the Cry toxins.

Residue Chemistry

As indicated previously, an exemption from the requirement of a tolerance has already been issued for this use. Residue chemistry data were not required because of the apparent lack of mammalian toxicity of this active ingredient. In the acute mouse oral toxicity study, the CryIA(c) δ-endotoxin was shown to have an LD_{50} greater than 4200 mg/kg. When proteins are toxic, they are known to act via acute mechanisms and at very low dose levels [Sjobald, Roy D., et al. "Toxicological Considerations for Protein Components of Biological Pesticide Products," Regulatory Toxicology and Pharmacology 15, 3-9 (1992)]. Therefore, since no significant acute effects were observed, even at relatively high dose levels, the CryIA(c) δ-endotoxin is not considered acutely or chronically toxic. (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-pesticide 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 triggered by significant acute effects in studies such as the mouse oral toxicity study. The acute oral mouse toxicity study for this product would not trigger the studies in Tiers II and III. In addition, studies summarized in Section C., Environmental Fate indicate that Btk protein was reduced to undetectable levels in cottonseed meal after processing. No detectable levels were found in refined oil at a level of detection of 1.3ppm.

The genetic material necessary for the production of the *Bacillus thuringiensis* CryIA(c) δ-endotoxin are the nucleic acids (DNA) which comprise: (1) genetic material encoding the CryIA(c) δ-endotoxin and (2) its regulatory regions. "Regulatory regions" are the genetic material that control the expression of the genetic material encoding the CryIA(c) δ-endotoxin, such as promoters, terminators, and enhancers. As stated above, no mammalian toxicity is anticipated from dietary exposure to the genetic material necessary for the production of the *Bacillus thuringiensis* CryIA(c) δ-endotoxin in cotton. Therefore, no residue data are required in order to grant an exemption from the requirements of a tolerance for the plant-pesticide, *Bacillus thuringiensis* CryIA(c) δ-endotoxin and the genetic material necessary for its production in cotton.

C. ENVIRONMENTAL FATE

The sites and levels of expression of the CryIA(c) delta endotoxin in cotton have been determined. The delta endotoxin is detectable in leaves, seeds, and whole plant assays. A total of 1.44 grams of protein would enter the soil per acre based upon

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estimates of 60,000 plants per acre. Btk protein was undetectable in cottonseed meal, and was present only at or near the level of detection in pollen and below the level of detection in nectar.

While gene transfer is not a problem throughout most of the areas where cotton is produced, due to a lack of sexually compatible relatives, there is some wild or feral cotton for which gene transfer is a concern. BPPD is therefore recommending that the containment provisions associated with the original limited registration be retained for cotton produced in the geographic areas where gene transfer is a possibility.

1. **Gene Expression:** Test substances were cotton lines 531 and 931. Six locations in Mississippi, Louisiana, Texas, Georgia, Arizona, and Alabama were used for field expression studies. Proteins in leaf, seed, whole plant, cottonseed meal and refined cotton seed oil were analyzed.

Expression level ranges were identified by validated ELISA procedures. Reported mean Btk protein expression levels from field grown plants ranged from 1.10 to 2.04 ug per gram of fresh leaf tissue and from 0.49 to 1.62 ug per gram fresh seed tissue.

Based upon planting rates of 60,000 plants per acre and, a total of 1.44 grams of Btk protein would enter the soil per acre due to post harvest incorporation of the plants into the soil.

Btk protein was reduced to undetectable levels in cottonseed meal after processing. No detectable levels were found in refined oil at a level of detection of 1.3ppm.

Greenhouse studies indicate that Btk protein is expressed in pollen (11.5 ng/gram) at a level of detection of 8.0 ng/gram, and is below the level of detection in nectar (<1.6ng/gram).

2. **Anaerobic Soil Degradation:** This study demonstrated a loss, following soil incorporation, in activity of cotton produced Btk endotoxin against a susceptible insect, the tobacco budworm Heliothis virescens. However, precise half-life and DT_{90} values could not be calculated due to the conduct and outcome of the experiment. This study is not a requirement for the proposed registration, as cotton is not typically grown under anaerobic conditions.

3. **Gene Transfer:** Domestic cotton, Gossypium hirsutum is an allotetraploid. There are no identified non-cotton plants which are sexually compatible to cultivated cotton. Wild and/or feral cotton does exist in some locations. There are three sites in the United States where wild/feral Gossypium (cotton) occurs naturally.

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G. thurberi occurs in the mountains of Southern Arizona. It is geographically and temporally isolated from domestic cotton. Hybridization is possible in the laboratory. Notwithstanding, any gene exchange between plants of G. hirsutum and G. thurberi would result in triploid, sterile offspring.

G. tomentosum occurs in Hawaii. As G. tomentosum may bloom at the same time as domestic cotton, there is no guarantee of either geographic or temporal isolation. G. tomentosum is tetraploid, and introgression has been claimed for what one author considered a hybrid of G. barbadense X G. tomentosum. For these reasons, BPPD recommends retaining the containment provisions of the original plant propagation registration for cotton expressing the CryIA(c) delta endotoxin grown in Hawaii. In response to concerns about gene transfer in Hawaii, Monsanto has submitted revised labelling incorporating the following restrictions:

"Not for commercial sale or use in Hawaii. Test plots or breeding nurseries established in Hawaii must be surrounded by either 12 border rows of non-transgenic cotton if the plot size is less than 10 acres or 24 border rows if the plot is over 10 acres and must not be planted within 1/4 mile of Gossypium tomentosum."

Feral G. hirsutum occurs in southern Florida in the Everglades National Park and the Florida Keys. Cotton is not grown commercially in these areas at this time, but the containment provisions of the initial registration must continue for areas in Florida where feral cotton occurs. Wild cotton is a concern as its potential role in the spread of resistance in Florida (with intensive vegetable production)is unknown. The PRMW report elaborates on this issue. Monsanto has submitted revised labelling restricting the use of Bt cotton in Florida to those sites North of Tampa (Route 60). BPPD is satisfied that this labelling will mitigate concerns for gene transfer to wild cotton.

D. ECOLOGICAL EFFECTS

1. **Avian Dietary LC50**. This study demonstrated that ground cottonseed expressing 0.9 ng Btk protein /g fresh wt is practically nontoxic to northern bobwhite quail when fed at 10,000 ppm in the diet for 5 days.

2. **Non target Insects**. Purified Btk endotoxin was practically nontoxic to the parasitic wasp, Nasonia vitripennis, green lacewing larvae, honeybee larvae, honeybee adults, and adult ladybird beetles when fed at 1,700 and 10,000 times the levels found in pollen and nectar.

Ecological Effects Assessment

The planting of cotton expressing the CryIA(c) endotoxin would not have detectable deleterious effects upon beneficial insects consuming pollen or nectar, or upon upland birds consuming cotton seed.

Studies on the effects of invertebrate soil organisms are not required. It was originally thought that since long-term exposure of soil organisms is possible when crop residues are incorporated or left upon the soil surface, EPA would require studies evaluating effects upon the representative soil organisms Collembola and earthworms. One of EPA's reasons for requiring the nontarget soil invertebrate tests (earthworm and Collembola) was the concern that adverse effects on these species would cause a build up of plant detritus in cotton fields. However, in reconsideration, EPA discovered that the long term soil use of highly toxic chemical insecticides, such as aldicarb, terbufos, phorate and carbofuran, which probably have long term effects on those species has not resulted in the build-up of plant detritus in cotton based upon available information on current routine agronomic practices. Thus Bollgard cotton, which is expected to have less impact on these species than the highly toxic chemical pesticides, should not result in any increased build up of plant detritus. Supporting thae conclusion, Monsanto has submitted data which indicate that delta endotoxin production ceases at senescence, allowing some time for protein degradation prior to harvest. Additionally, as the environmental fate data indicate that only 1.44 grams of Btk protein per acre would enter the soil as a result of post harvest incorporation of Bt cotton, and such proteins are known to degrade rapidly, the potential for effects to non-target soil organisms is not anticipated. Thus, an observable deleterious effect on the soil ecosystem resulting in a build up of plant detritis is not expected to result from the growing of CryIA(c) δ-endotoxin-containing cotton plants such as the proposed product.

<u>Endangered Species Considerations</u>

A Biological Opinion from the Department of the Interior Fish and Wildlife service was issued on December 18, 1986, concerning the possible effect of foliar spray of *Bacillus thuringiensis* subsp. *kurstaki* (*Bt*) on threatened and endangered species. Based on the difference in exposure scenarios between foliar spray and expression of *Bt* in cotton plants, EPA believes that the Biological Opinion is inapplicable, and that reinitiation of consultation is not required.

This 1986 Biological Opinion stated that the reregistration of the microbial pesticide, *Bacillus thuringiensis* subsp. *kurstaki*, used to control lepidopterous pests on agricultural crops, ornamental crops, forest trees, and all other crops listed in 40 CFR 180.34 is likely to jeopardize the continued existence of the Kern primrose sphinx moth, Lange's metalmark butterfly, Smith's blue butterfly, El Segundo blue butterfly, Oregon silverspot butterfly, Mission blue butterfly, San Bruno elfin butterfly, Lotis blue butterfly, and Schaus swallowtail butterfly. Critical habitat of any of the above-listed species will not be adversely modified or destroyed. The Dept. of Interior document also specified that *B. thuringiensis* subsp. *kurstaki* or any formulations thereof should not be applied to crops and forests near or in the habitats of the species listed and provided the locations of their habitats. This Opinion applies to foliar spray, bait or granular applications of microbial *Bt* products produced by fermentation, concentration and subsequent formulation, and not plant-pesticides. Based on the difference in exposure scenarios between foliar spray and expression of *Bt* in cotton plants, EPA believes that the Biological Opinion in inapplicable, and that reinitiation of consultation with the Dept. of Interior is not required. Expression in cotton will not result in spray drift and only those insects that feed on cotton will be exposed.

Although cotton pollen containing the CryIA(cb) δ-endotoxin can drift out of fields, such pollen, at relatively very high dosages, was not toxic to the test species representative of organisms likely to be exposed to such pollen when cotton plants containing the cryIA(c) gene are grown. 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 that any endangered/threatened species will be adversely affected by pollen containing the CryIA(c) δ-endotoxin.

In addition, because EPA is imposing conditions for geographic areas (Hawaii and Florida) that have sexually compatible wild or weedy relatives of cotton, the CryIA(c) δ-endotoxin gene cannot escape into plants on which endangered/threatened species feed on in these areas.

Because EPA expects that in most cases, no listed endangered species of Lepidoptera will be exposed to the *Bt* protein expressed in cotton plants, and because the most probable exposure scenario does not appear to affect listed species, EPA believes that this action will have no effect on listed species.

E. RESISTANCE MANAGEMENT

1) <u>Resistance Management Assessment</u>

The Pesticide Resistance Management Workgroup's (PRMW) has completed a technical evaluation of the resistance management strategy voluntarily submitted by Monsanto Company to help mitigate the development of tobacco budworm (TBW) (Heliothis virescens (Fabricius)), bollworm (CBW) (Helicoverpa zea (Boddie)) and pink bollworm (PBW) (Pectinophora gossypiella (Saunders)) resistance to the CryIA(c) delta endotoxin produced in Bollgard® cotton. The PRMW has provided recommendations of what would be necessary to provide a long-term resistance management strategy for mitigating the development of resistance, primarily for the CBW, PBW and TBW.

The PRMW has identified the following concerns associated with the development of resistance in lepidopteran pests exposed to plant-pesticides produced in crops such as Bollgard® cotton.

(1) Impact of season-long selection pressure by the CryIA(c) delta endotoxin on CBW, PBW, TBW and other lepidopteran pests in large scale, commercial plantings of cotton with the CryIA(c) delta endotoxin. In general, conventional cotton insecticides have relatively short-lived efficacy and rapidly degrading residues when compared to the sustained efficacy of Bollgard® cotton. Thus, the selection pressure on the target pests is quite different between plant-pesticides and conventional insecticides.

(2) The potential for these lepidopteran pests to develop resistance to Bt delta endotoxins (CryIA(c)) in Bollgard® cotton and the implications for the control of these pests on numerous other crop hosts. Some of these other crops also rely on Bt plant-pesticides or foliar Bts.

However, the PRMW believes it may be possible to at least mitigate or delay resistance. This idea is supported by the inadvertent "refugia" created when synthetic pyrethroid registrations were largely limited to use on cotton. The CBW, with its broad host range, did not become resistant. However, the TBW with a narrower host range did develop resistance to the synthetic pyrethroids.

Monsanto has addressed all of the general elements of a resistance management program, some in considerable detail and others superficially. These elements include pest biology, Bt dose deployment, refugia, monitoring for resistance, susceptible nontarget Lepidoptera pests, cross resistance to related Bt toxins, IPM fit, grower education, and development of alternative pesticides with different modes of action.

2) Resistance Management Risk Mitigation

The PRMW concludes that the Monsanto Company's resistance management plan has many workable elements, but it is necessary to modify the plan and have specific elements finalized to adequately manage tobacco budworm (TBW), bollworm (CBW) and pink bollworm (PBW) resistance to the CryIA(c) delta endotoxin produced in Bollgard® cotton. There are many unanswered resistance management questions concerning Bollgard® cotton, particularly in light of the differences that exist between the lepidopteran pest complexes and cropping practices within the four major USA cotton belt growing areas (i.e., Southeast, Mid-south, Southwest, West).

The PRMW concludes that the following data and programs are necessary within the next 5 years, to determine how to mitigate the development of resistance to the CryIA(c) delta endotoxin:

(1) Conduct research to determine the effectiveness of Monsanto's proposed "structured" refugia options and possibly other resistance management options, on farms planted with Bollgard® cotton.

(2) Develop, implement, and evaluate the monitoring program for the development of resistance (reduced percent control) in TBW, CBW, PBW and the other nationally or regionally important lepidopteran pest species (i.e., cotton leafperforator, saltmarsh caterpillar, European corn borer, cabbage looper, soybean looper) to the CryIA(c) delta endotoxin;

(3) Review literature and conduct research on pest biology for TBW, CBW, and PBW including development, mating behavior, survival and fecundity on the key regional non-cotton hosts and adult movement to and from cotton and other hosts;

(4) Determine rate of gene movement between populations of TBW, CBW, and PBW across the cotton producing states;

(5) Undertake further quantification of Bt expression and efficacy in Bollgard® and any new gene selections for the TBW, CBW, PBW and other lepidopteran pests on cotton;

(6) Provide annual reports of this research and resistance monitoring to the Agency;

(7) Provide annual reports to the Agency of the acreage and location of the use of Bollgard® cotton, by variety.

These conclusions are, in part, based upon the understanding that other Bt-cotton products producing the CryIA(c) delta endotoxin to control these lepidoptera would be subject to the same terms, conditions, data requirements and acreage limitations as Bollgard® cotton. Initially, Bollgard® cotton producing the CryIA(c) delta endotoxin may only be planted in relatively

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limited acreage. Therefore, the selection pressure on the target Lepidopteran pests for resistance may also be limited at first. The PRMW estimates that this initial period may be at most 5 years. Registrations of other CryIA(c) delta endotoxins produced in cotton and other crops could increase the acres producing Bt delta endotoxins and reduce the acres of non-Bt cotton and other host refugia available to help in resistance management. Resistance management strategies will depend upon relative numbers of acres that may serve as refugia and local patterns of planting CryIA(c) and other Bt delta endotoxin producing crops relative to non-Bt endotoxin producing crops. In addition, if field corn producing the CryIA(c) delta endotoxin in kernels to control the corn earworm (CEW)/CBW) are to be registered, this would likely increase the selection pressure for resistance in CEW/CBW, unless its use is limited to non-cotton producing states.

The PRMW suggested a five year conditional registration for the CryIA(c) delta endotoxin produced in Bollgard® cotton. There are many outstanding data gaps that do not allow the PRMW to make any definitive conclusions about the potential success of the proposed Monsanto resistance management strategy. Because of the high degree of uncertainty and the unknown ramification of season-long exposure of the target insect pest complexes to the CryIA(c) delta endotoxin, the PRMW believes that additional research data, a specific monitoring plan including the development of discriminating doses for the TBW, PBW, and CBW, field validation of "structured" refugia and efficacy, and annual reporting are required before a conclusion can be made about the potential success of the proposed resistance management strategy.

As a result of the PMRW review, the following conditions are being imposed on the registration. Monsanto company has agreed to these conditions in writing:

1. This registration will automatically expire on midnight January 1, 2001. EPA will reevaluate the effectiveness of Monsanto's resistance management plan before January 1, 2001, and decide whether to convert the registration to a non-expiring (and/or unconditional) registration.

2. Monsanto Company will submit, by June 1, 1996, literature and information on target pest biology and ecology. Such information will relate to a literature review on the local biology (e.g. interfield movement and behavior, and the importance of development rate, survival and fecundity on non-cotton hosts) of the cotton bollworm(CBW), the tobacco budworm(TBW), and the pink bollworm(PBW).

3. Monsanto will submit, by January 31, 1998, research data concerning such target pest biology, including data regarding the effect of different hosts on the development, survival and

fecundity of these pests in order to assess the significance of selected non-cotton hosts as refugia.

4. Monsanto company will submit, by April 1, 1996, a protocol for determining the likelihood of cross resistance to other B.t. endotoxins. Data evaluating the potential for cross resistance must be submitted by January 31, 1998.

5. Monsanto company will, by March 1, 1996, submit a plan for a workable resistance monitoring program, and submit the existing data for baseline susceptibility for PBW, CBW, and TBW. Where the information does not already exist, data must be submitted which provide baseline susceptibility and discriminating doses for these pests by January 1, 1997. The monitoring plan should establish specific locations in selected states that will be monitored annually at a central laboratory location, with duplicate sample collections sent to a second lab for confirmation. Monsanto will also agree (as per the company proposal) to 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 (PBW, CBW, and TBW) as well as for cabbage looper, soybean looper, saltmarsh caterpillar, cotton leafperforator and European corn borer. Monsanto will also indicate in this plan how resistance management strategies would be altered should resistance be detected. A preliminary report on results of this monitoring must be submitted to the Agency annually by November 1 each year and a final report will be submitted to the Agency annually by January 31 each year for the duration of the conditional registration.

6. Monsanto Company will submit annual reports (by Nov. 1st each year) on the use of Bollgard® cotton by acreage, locality (state and region, if applicable), and variety.

7. Monsanto will continue the development and distribution of 1) educational materials for growers, 2) the technical bulletin on the use of the product 3) materials on how to monitor and report resistance.

8. Monsanto will continue to investigate the influence of B.t. cotton on secondary lepidopteran pests (cabbage looper, soybean looper, saltmarsh caterpillar cotton leafperforator and European corn borer).

9. Monsanto will submit data relevant to the expression and degradation of the CryIA(c) endotoxin in various plant parts in correlation with susceptible doses for lepidopteran pests, by January 1, 1998.

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V. POTENTIAL BENEFITS

Based upon the submitted data, Bt cotton is as effective (or in some situations more effective) when compared to available registered alternatives for the control of the bollworm, tobacco budworm, and the pink bollworm. While not quantified by the submission, it may also be as efficacious as available registered alternatives for the cotton leafperforator and the saltmarsh caterpillar (which are not claimed as controlled pests on the proposed label). In cotton growing areas where resistance by the target pests to the existing chemical alternatives has been demonstrated, significant yield increases have been reported. While past the official comment period, the Agency continues to receive testimonials of enhanced yield and product performance from growers and state universities.

Because of this efficacy, using Bt cotton should reduce the number of sprays for these lepidopterous pests in geographic locations where they are a production factor. Reduction in sprays in the submitted data were from 4 to 9 less sprays per year, depending on the population density of non-lepidopteran cotton pests. Registered alternatives include organophosphates, synthetic pyrethroids, and amidazoles which have greater potential for adverse effects to man and the environment. Therefore the registration of the proposed Bt cotton will reduce the number of applications of more hazardous alternatives.

Data from the ecological effects package have indicated no effects expected on beneficial parasites and predators. The range of activity for the CryIA(c) protein is well known, and does not include insect orders in which parasites and predators are typically classified. Therefore, this product should fit well into existing IPM programs. For this reason, the use of this product may further reduce the use of more toxic chemical alternatives.

Benefits from the pesticide being expressed by the plant rather than applied using humans and equipment are also apparent. These benefits include:

1. Savings from fuel, equipment, and labor costs associated with the reduction in applications.

2. Elimination of the potential for applicator and farm worker exposure associated with the use of more toxic compounds.

3. Reduced potential for human and environmental hazards from the elimination of drift into non-target areas.

4. Growers would be less dependent on weather for insecticide applications.

5. Adverse effects on target organisms should be reduced because the only organisms able to receive a dose are those feeding on the crop.

VI. LABELING

In order to provide for containment of the gene and appropriate refugia to mitigate the development of resistance as described in the Environmental Fate and PRMW reviews, Monsanto has submitted revised labeling incorporating the following provisions:

1. In Florida do not plant south of Tampa, (Florida Route 60). Not for commercial sale or use in Hawaii. Test plots or breeding nurseries established in Hawaii must be surrounded by either 12 border rows of non-transgenic cotton if the plot size is less than 10 acres or 24 border rows if the plot is over 10 acres, and must not be planted within 1/4 mile of Gossypium tomentosum.

2. The grower guide which accompanies the seeds containing the plant-pesticide includes provisions for growers to read and follow required resistance management practices, unless they are under specific contract to Monsanto (Delta Pineland Company and/or Hartz Seed Company) to produce cotton containing the bollgard gene for future planting. Growers are required to choose and implement one of the following refuge options:

A. For every 100 acres of cotton with the Bollgard gene planted, plant 25 acres of cotton without the Bollgard gene that CAN be treated with insecticides (other than foliar B.t.k. products) that control the tobacco budworm, cotton bollworm and pink bollworm.

B. For every 100 acres of cotton with the Bollgard gene planted, plant 4 acres of cotton without the bollgard gene that CANNOT be treated with acephate, amitraz, endosulfan, methomyl, profenofos, sulprofos, synthetic pyrethroids, and/or B.t.k. insecticides labelled for the control of tobacco budworm, cotton bollworm, and pink bollworm. This cotton must be managed (fertility, weed control and management of other pests) in a similar manner as bollgard cotton.

NOTE: If cotton with the Bollgard gene exceeds 75% of the total amount of the cotton planted in any single county or Parish in any year, growers in that county or Parish choosing option B the following year will be required to plant the 4% refugia within one mile of the respective Bollgard cotton field. Monsanto will notify growers who are in an affected county or Parish. If EPA grants a registration for cotton containing the B.t.k. insect control protein with a similar mode of action as the CryIA(c)

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insect control protein to another company(s), the EPA will determine when the total cotton within a county or Parish exceeds the 75% level. This determination will be made using annual reports or planted acreage submitted by the registrants. Should EPA determine the combined acreage of cotton containing the B.t.k. insect control protein exceeds 75%, they will inform the registrants by January 1, that the refuge must be planted within one mile of the respective Bollgard cotton or other B.t.k. cotton fields.

VII. RATIONALE FOR RECOMMENDATION

Pursuant to FIFRA section 3(c)(7)(B), EPA may conditionally amend the registration of a pesticide to permit an additional use if two criteria are fulfilled: 1) the applicant has submitted satisfactory data pertaining to the proposed new use; and 2) amending the registration in the manner proposed by the applicant will not significantly increase the risk of any unreasonable adverse effect. BPPD believes that both these criteria have been fulfilled.

A. ADDITIONAL USE REGISTRATION UNDER FIFRA 3(c)(7)(B)

The applicants have submitted or cited data to satisfy the first criterion for conditional registration under FIFRA 3(c)(7)(B). Monsanto has submitted data pertaining to the proposed additional use of the product in cotton, including the incremental risks that would result from approval of the applications. The human health effects data and nontarget organism effects data are considered complete and no potential adverse effects are foreseen in these areas. In addition, BPPD believes that the applicants have provided sufficient data to characterize the incremental risks associated with the development of resistance. The applicant has agreed to appropriate conditions and limitations of the use of the product to mitigate these risks. In conclusion, amending the existing registration by accepting the amendments 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 is satisfactory, it is not sufficient to support an unconditional amendment under FIFRA 3(c)(5). Additional data are necessary to evaluate the risk posed by the development of resistance to Cry δ endotoxins that is associated with generic use of these products. As discussed in more detail in section III above, the introduction of these products for any wide-scale use poses the risk that pests, such as the bollworm, will develop resistance to many different *Bt* microbial pesticides that are used on a wide variety of crops. BPPD believes that the applicants have submitted sufficient data to allow the Agency to determine that the applicants' plans to manage this risk will be workable for 5 years following initial commercial use of these products. Additional data, however, are necessary to determine how to effectively reduce the risks associated with resistance beyond that initial period. Consequently, BPPD recommends imposing the data requirements specified earlier in this Decision Document in section III E. 2).

BPPD also believes that the second criterion for a FIFRA 3(c)(7)(B) conditional registration has been fulfilled 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 this products 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 economic, social, and environmental costs and benefits of the additional use as restricted by the terms and conditions of registration.

The proposed new use of this product on greater acreage poses the risk of the development of multiple- and cross-resistance in certain Lepidopteran pests of cotton. As a result, pests could develop resistance to certain microbial *Bt* pesticides that are applied to both cotton and other crops and reduce the utility of such products. 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. A review of the reports published by the USDA National Agricultural Statistics Service (Agrichemical Usage Vegetables, Field Crops) indicates that significant *Bt* usage occurs not only for cotton, but also for vegetable crops such as broccoli, cabbage, celery, eggplant, lettuce, bell peppers, spinach, and tomatoes. Chemicals registered for the target pests of cotton which are also registered for these vegetables include synthetic pyrethroid (fenvalerate, permethrin, fluvalinate, bifenthrin and cyfluthrin) organophosphate (chlorpyrifos, monitor, guthion) and carbamate (methomyl) pesticides. 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.

This registration should provide substantial benefits to cotton producers in the form of increased yields resulting from the control of damage caused by PBW, CBW, and TBW, and possibly other cotton pests. The registration of this product should also reduce the use of more toxic and environmentally hazardous chemical pesticides. As no applications other than planting are

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required , the hazards to mixers, loaders, and workers entering treated fields are reduced. The nature of the plant-pesticide protein being expressed in the cotton also eliminates the potential for drift to non-target sites. The range of biological activity for the CryIA(c) protein is well known, and does not include beneficial parasites and predators that occur in cotton fields. Submitted data indicate no effects on the beneficial insects tested. Therefore the use of this product should fit well into existing IPM programs, further reducing the use of more toxic traditional chemical pesticides.

The risks from pesticide resistance are substantial and BPPD has concluded that the risks, if unchecked, could outweigh the benefits of the proposed new use. However, the terms and conditions of registration that are recommended in Section III and Section VI. of this Decision Document (requiring specific plans for refugia and acreage limitations) 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. This registration will expire automatically on January 1, 2001. Before that time EPA can re-evaluate whether the resistance management plan has been effective. In the interim, the registrant must conduct a grower education program directed at increasing grower awareness of resistance management; conduct monitoring to help detect the development of resistance to the CryIA(c) δ-endotoxin; conduct research to determine how to develop an effective long-term resistance management plan; and implement an EPA-approved structured refugia system.

VIII. RECOMMENDATION

The submitted data in support of this amended registration under section 3(c)(7)(B) of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) have been reviewed and determined to be adequate. Studies and information regarding resistance management are included in the terms, conditions, and limitations of this registration. These data requirements will apply to both the existing seed propagation use and the proposed new use for commercial production. Amending the existing registration will not cause an increase in significant adverse effects to man or the environment, either as a result of exposure to non-target organisms or from the potential for the development of resistance.

Furthermore, the benefits of the new use pattern have been well established and the terms, conditions, and limitations imposed by this registration mitigate the risks posed from the potential pest resistance to *Bt*. Therefore, the potential benefits outweigh potential risks, i.e. from the development of resistance.

Based on the data submitted by the registrants and reviewed by OPP staff, Biopesticides and Pollution Prevention Division recommends that Monsanto's plant-pesticide product containing the active ingredient *Bacillus thuringiensis* CryIA(c) δ-endotoxin and the genetic material necessary for its production in cotton be CONDITIONALLY REGISTERED for food and feed use under 3(c)(7)(B) of FIFRA.

CONCUR: ______________________

NONCONCUR: ______________________

DATE: 10/31/95