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

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OFFICE OF PREVENTION, PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Consideration of Section 3(c)(7)(B) Conditional Amendments for Ciba Seeds' and Mycogen Plant Science's Plant-Pesticide *Bacillus thuringiensis* CryIA(b) δ-Endotoxin and the Genetic Material Necessary for Its Production (Plasmid Vector pCIB4431) in Corn. (EPA File Symbols 66736-1 & 68467-1)

-DECISION MEMORANDUM-

FROM: Janet L. Andersen, Acting Director
Biopesticides and Pollution Prevention Division

TO: Daniel M. Barolo, Director
Office of Pesticide Programs

I. ISSUE

Should the Agency: (1) conditionally amend the existing FIFRA §3(c)(5) registration for limited plant propagation use to permit an additional use of the product in field corn pursuant to FIFRA §3(c)(7)(B). The active ingredient in this pesticide product is the *Bacillus thuringiensis* CryIA(b) δ-endotoxin and the genetic material necessary for its production (plasmid vector pCIB4431) in corn. "Genetic material necessary for its production" means the genetic material which comprise (1) genetic material encoding the CryIA(b) δ-endotoxin and (2) its regulatory regions. "Regulatory regions" are the genetic material that control the expression of the genetic material encoding the CryIA(b) δ-endotoxin, such as promoters, terminators, and enhancers. The limitations currently placed upon the use of the products 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.

II. SUMMARY AND REGULATORY BACKGROUND

Ciba Seeds and Mycogen Plant Sciences have both submitted applications to amend their seed increase/hybrid production registrations to expand these registrations to full commercial use for field corn while retaining the seed increase/hybrid production uses for sweet corn and popcorn. Ciba Seeds has also

submitted petitions requesting exemptions from the requirement of a tolerance for 1) *Bacillus thuringiensis* CryIA(b) δ-endotoxin and the genetic material necessary for its production (plasmid vector pCIB4431) in corn and 2) the plant-pesticide inert ingredient phosphinothricin acetyltransferase (PAT) and the genetic material necessary for its production (plasmid vector pCIB3064) in corn. Substances and the genetic material encoding them, that are used to assist in the identification of plants or plant cells containing the active ingredient are considered inert ingredients for plant-pesticides. These substances are referred to as "markers". PAT confers resistance to the herbicide gluphosinate and is used as a marker for corn plants producing the CryIA(b) δ-endotoxin. EPA published a receipt of application for registration from Ciba Seeds and Mycogen Plant Sciences on January 13, 1995 in the Federal Register and the notices of filing regarding the petitions for exemption from the requirement of a tolerance were published in the Federal Register February 1, 1995 (60 FR 6092).

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 limited registrations that allow Ciba Seeds (Ciba) and Mycogen Plant Sciences (Mycogen) to produce seed for seed increase and hybrid production, but that are limited in scope and duration.

The Biopesticides and Pollution Prevention Division (BPPD), the Biological and Economic Analysis Division (BEAD), and the Pesticide Resistance Management Workgroup (PRMW) have evaluated the data submitted by Ciba and cited by Mycogen and, based on these data and other relevant information, believes that the products will perform their intended function and that the applicants have submitted/cited satisfactory data pertaining to the proposed additional uses and amending the registrations in the manner proposed by the registrants would not significantly increase the risks of any unreasonable adverse effects to humans, nontarget organisms, or the environment from the food and feed use of these products in field corn. 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 in the public interest. The PRMW has reviewed the pesticide resistance management plans and has identified certain terms and conditions necessary to the potential for development of insect resistance to *Bacillus thuringiensis*.

The amended registrations for food and feed use of these products in field corn, if granted, would be conditional under section 3(c)(7)(B) of FIFRA. The Agency is imposing terms and conditions as outlined in section IV(D) of this document to address resistance management concerns. The existing FIFRA § 3(c)(5) registrations for seed propagation of sweet corn and popcorn would remain and continue to be subject to the current terms and conditions.

These conditions and terms are being imposed to address concerns that insects will develop resistance to *Bt* plants and sprays which can be used on corn, cotton, and a variety of vegetable crops. The conditionally required data were not listed as a requirement for this active ingredient prior to the date of the submission by Ciba and Mycogen.

III. PUBLIC COMMENTS TO NOTICE OF RECEIPT AND SAP MEETING

A. PUBLIC COMMENTS TO NOTICE OF RECEIPT

Four individuals or organizations provided written comment on the notice of receipt for the proposed registration. The National Corn Growers Association and American Seed Trade Association comments were positive in nature, noting the potential benefits of the plant-pesticide. Two commenters expressed concerns regarding emergence of resistance to the pesticide in the target pest:

(1) Dr. David Andow, Department of Entomology and Dr. Donald Alstad, Department of Ecology, Evolution, and Behavior of the University of Minnesota present 3 concerns: (1) resistance to the CryIA(b) δ-endotoxin produced in Event 176-derived hybrids would result in the loss of *Bt* microbials because of cross-resistance or multiple resistance, (2) the assumption that if resistance develops, it will be due to a single, rare gene and this gene will be fully recessive is flawed, therefore a resistance management strategy needs to be adopted and (3) a resistance management strategy using the Andow and Alstad model of employing a "50-50 patchwork" of early- and late-planted fields is recommended.

(2) Dr. Rick Roush, Department of Entomology, Cornell University presents several concerns: (1) a minimum 20% "structured" refuge on every farm is considered to be fundamental to managing resistance to *Bt* δ-endotoxins expressed in plants, (2) ECB, and other corn pest, resistance may have a deleterious effect on beneficial insect populations, (3) resistant alleles may not necessarily have a random distribution, and (4) *Bt* toxin resistance may occur in corn insects other than ECB. Because of these concerns, Roush recommended that EPA reject the registrant's application for the registration of the CryIA(b) δ-endotoxin in Event 176-derived hybrids.

Agency Response

The Agency recognizes the risks associated with the development of resistance to the CryIA(b) δ-endotoxin and the need for a long-term implementable resistance management strategy. Ciba Seeds and Mycogen Plant Sciences have provided a resistance management plan in their original October 18, 1994 document. In addition, they have provided further refinements to their resistance management plan (May 31, 1995 and June 7, 1995). It appears that both corporations are committed to implementing a resistance management plan. OPP's Pesticide Resistance Management Workgroup's detailed technical analysis of Ciba Seeds' and Mycogen Plant Science's resistance management strategy is discussed in the support document entitled: "Technical Evaluation of Ciba Seeds'/Mycogen's Resistance Management Plan: "Assessment and Management of European corn borer (ECB) Resistance to Transgenic Corn (Maize) Containing the Event 176 Insect Control Protein" (October 18, 1994) and Refinements to the Plan (May 31, 1995 and June 7, 1995)".

1) Comments on Alstad and Andow

Potential for Loss of Use of Microbial *Bt* Pesticides

The Agency agrees with Andow and Alstad that cross-resistance and/or multiple resistance could develop to the CryIA(b) δ-endotoxin such that there could be some deleterious impact on *Bt* microbial pesticides without a resistance management strategy. The Agency has made several recommendations for further research and actions to prolong the development of resistance to CryIA(b) (discussed below in the recommendations section).

Single, Rare *Bt* Resistance Gene Hypothesis

The Agency agrees with Alstad and Andow that it is premature to assume that if resistance develops it will be due to a single, rare gene and this gene will be fully recessive. Resistance may actually be the result of the contributions of several genes.

This is especially true if the Event 176 corn plants producing the CryIA(b) δ-endotoxin fail to kill 100% of susceptible insects. It is possible that the single locus, single allele analysis underestimates the likelihood that resistance will evolve. Thus, the Agency agrees with Andow and Alstad that there is a critical need to develop and implement a resistance management strategy for the CryIA(b) δ-endotoxin produced in Event 176-derived hybrids by Ciba Seeds and Mycogen Plant Sciences.

Fifty-fifty Patchwork Model

The Agency believes that the "50-50 patchwork" model proposed by Andow and Alstad should be investigated as a resistance management option in addition to other types of "structured refugia". The Agency concludes that this model must be validated in the field in order to determine its actual effectiveness.

2) Comments on Roush

Structured Refugia

The Agency agrees with Roush that "structured" refuges appear to be fundamental to managing resistance to *Bt* δ-endotoxins expressed in plants. The major issues are how to structure refuges and how large a refuge is needed after the first several years of commercialization, when the acreage devoted to corn producing the CryIA(b) or CryIA(c) δ-endotoxins will be of a significant size to warrant structured refugia. There is no agreement among research scientists and industry as to what is an adequate size. The values for adequate "block" refuge size primarily range from 20% to 50% of the acreage per farm being proportioned into corn producing *Bt* plant-pesticides and conventional corn blocks. The Agency believes that the minimum adequate size or structure for a "structured" refuge cannot be determined until additional research is conducted. The answer is likely to be different for the high density corn acreage of the midwestern corn belt versus the low density acreage of the fringe states which may be part of the cotton belt.

Impact of ECB Resistance on the Natural Enemy Complex

Although the Agency agrees with Roush that ECB resistance is a concern, the Agency does not agree with his argument that the reduction in the natural enemies will result in significantly higher ECB populations in the future. The predictive models referred to by Roush are unproven. Ciba Seeds' and other investigator's field plots were too limited in size for the Agency to fully determine the impact on populations of natural

enemies (beneficial organisms) by *Bt* corn concentrated over large areas such as in the corn belt.

ECB is an introduced pest species, as are most of the more important beneficial species that attack it. These natural, biological controls generally do not exert satisfactory control over this pest. The economic impact of ECB is neither uniform throughout its distribution nor over time in any one location. Also, it is unlikely that *Bt* corn varieties will become the predominate ones wherever corn is grown.

The decline in numbers of the insect parasites including *Lydella thompsoni*, the example mentioned by Roush, was very probably due to the introduction and establishment of *Nosema pyraustae*, a protozoan pathogen of the ECB. This reduction in natural enemy populations was extensive because *N. pyraustae* infected ECB larvae no matter what host plant the larvae were feeding upon. The CryIA(b) δ-endotoxin will be in corn, and not in the many other host plants that ECB develop on, such as potatoes, beans, beets, celery, dahlias, asters, gladioli, chrysanthemums, peppers, and many weeds. Thus, these other host plants could provide a reservoir for ECB larvae supporting populations of the natural enemies in question.

In addition, in the event ECB resistance develops, it will likely develop relatively slowly. As resistance develops in ECB populations, and the populations on corn slowly increase in numbers, so will the natural enemy populations which will be coming from the refugia of alternate host plants of ECB. Thus, there is not likely to be a significant rebound increase in ECB numbers over what was present before CryIA(b) δ-endotoxin was put into corn.

In the event that some beneficials are eliminated, in limited areas, they would likely migrate back into these areas, if the ECB became resistant and populations increased. The natural reestablishment of beneficial organisms could also be augmented by introduction from elsewhere in the USA or from their original habitats overseas.

Given the mobility of ECB and the substantial ECB refugia that will exist (multiple alternate hosts), it is not likely that either ECB or its pathogen, *Nosema pyrausta*, will be "eradicated". If ECB-resistance to CryIA(b) δ-endotoxin were to develop, it is expected that these resistant larvae would still be susceptible to *Nosema* infections. The scientific literature does not contain any information that would indicate a resistance mechanism that could confer ECB cross-resistance to both *Nosema* and CryIA(b) δ-endotoxin.

Probability for Resistance

The Agency agrees with Roush that resistance alleles may not have a random distribution and that resistant alleles with frequencies of even 10^{-5} may have a chance of becoming established in the absence of "structured" refugia.

Bt Resistance in Corn Insects Other than ECB

The Agency agrees with Roush that there is a potential of cross-resistance or multiple resistance between the various Cry I (also Cry IIA) δ-endotoxins for the ECB, and possibly other Lepidoptera on corn such as the southern cornstalk borer, *Diatraea crambidoides*; southwestern corn borer, *Diatraea grandiosella*, and the lesser cornstalk borer, *Elasmopalpus lignosellus*.

It is difficult to predict the likelihood or extent to which the corn earworm, *Helicoverpa zea*, will develop resistance to the CryIA(b) δ-endotoxin in corn. The corn earworm will be exposed to the CryIA(b) δ-endotoxin in southern states corn-growing areas where there is early season leaf feeding by corn earworm on whorl stage corn. The whorl stage leaves contain relatively high levels of the CryIA(b) δ-endotoxin. This possibility is minimal in Northern states e.g., feeding exists in Long Island on early season sweet corn.

However, later generations predominantly feed on silk and corn kernels, which contain levels of CryIA(b) δ-endotoxin at or below the level of detection. Thus, the later generations would not be exposed to CryIA(b) δ-endotoxin, and therefore, there would be no selection pressure for resistance.

Some Lepidoptera which primarily feed on corn have alternative hosts, such as CEW on cotton, where they pose an economic concern. The Agency is concerned about the ramifications of CEW resistance developing in insect populations that feed on both corn and cotton, and that this *Bt* resistance could negatively affect the utility of *Bt* foliar sprays on vegetables.

Recommendation to reject registration of CryIA(b) δ-endotoxin produced in Event 176-derived hybrids

Unless resistance management plans were implemented, Roush recommended that EPA reject the application to register CryIA(b) δ-endotoxin produced in Event 176-derived hybrids. As explained in section VI outlining EPA's conclusions on risks and benefits, amending these registrations to allow for full commercial use of field corn only with the stated terms and conditions for resistance mitigation described in section IV.D.2., the controlled use is such that no unreasonable adverse effects should result.

B. SAP MEETING AND RESULTING PUBLIC COMMENTS

A Science Advisory Panel (SAP) meeting was held on March 1, 1995, to allow for external scientific peer review and public participation in the decision process for *Bt* plant-pesticides in potatoes and some consideration of *Bt* plant-pesticides in corn and cotton. While the SAP report does not directly address *Bt* as expressed in corn, there appears to be nothing in the report which precludes this action. The SAP stated "Cotton and corn resistance management is potentially more complex than for potatoes." They further indicated that particular attention should focus on situations where *B.t.* genes are bred into 2 crop plant species which have one or more pest insects in common. In these situations, there is a potential for pests that have developed resistance on one crop producing a *B.t.* δ-endotoxin to spread the resistance to pest populations on a second crop producing the same *B.t.* δ-endotoxin.

In addition to comments received in response to the notice of receipt for this active ingredient, several comments regarding the Science Advisory Panel meeting that addressed *Bt* potato are relevant to this new use. In particular 7 commentors requested that EPA withhold its approval for any *Bt* plant-pesticides until adequate resistance management measures were in place. These requests were made largely on the basis that *Bt* plant-pesticides without effective resistance management plans could speed up the development of resistance in *Bt* sprays and eliminate these safer pesticides from the pest control options of the organic and sustainable agriculture farmer as well as other growers who employ *Bt*. The Agency shares these concerns and has placed a strong emphasis on resistance management in order to protect not only the *Bt* sprays, but also the use of *Bt* plant-pesticides.

IV. SCIENCE ASSESSMENT

The discussion that follows summarizes BPPD's, BEAD's, and the PRMW's reviews of the data available to the Agency on these products. A more detailed discussion of this assessment is provided in the Data Evaluation Records for the studies summarized below.

A. HUMAN HEALTH

1. CryIA(b)

PRODUCT ANALYSIS

Ciba Seeds submitted information which adequately described the truncated CryIA(b) δ-endotoxin as expressed in corn, along with data on the genetic material necessary for its production.

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Product analysis data were submitted to show that microbially expressed and purified CryIA(b) δ-endotoxin used for mammalian toxicological testing purposes is not significantly different than the δ-endotoxin expressed in the plant. The following assays were used to determine the similarity of the microbially expressed and purified CryIA(b) δ-endotoxin and that produced in corn: SDS-PAGE, western blots, amino acid sequencing, certain tests for post-translational modifications and insect bioactivity. These assays have demonstrated the truncated CryIA(b) δ-endotoxin expressed in corn and the tryptic digested CryIA(b) δ-endotoxin to be similar. The N-terminal amino acid sequences of both δ-endotoxins were found to be identical except that the plant produced δ-endotoxin had portions at the N-terminus deleted (perhaps due to internal plant proteases) and a higher bioactivity. These differences were not considered toxicologically significant since they are not expected to affect the activity of the δ-endotoxin in mammalian systems.

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(b) δ-endotoxin and the genetic material necessary for its production (plasmid vector pCIB4431) when used as a plant-pesticide in any corn plant.

The data Ciba Seeds submitted regarding potential health effects include information on the characterization of the expressed CryIA(b) δ-endotoxin in corn, 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 by Ciba Seeds support the prediction that the CryIA(b) 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(b) δ-endotoxin is not considered acutely or chronicly 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 corn. Production of microbial produced CryIA(b) 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(b) δ-endotoxin are the nucleic acids (DNA) which comprise (1) genetic material encoding the CryIA(b) δ-endotoxin and (2) its regulatory regions. "Regulatory regions" are the genetic material that control the expression of the genetic material encoding the CryIA(b) 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(b) δ-endotoxin in corn.

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. Ciba Seeds has submitted data to indicate that the CryIA(b) 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 corn kernels and is not detectable in finished silage) 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 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.

Submitted Data-

1. Acute Oral Toxicity of Bacterially Produced CryIA(b) δ-endotoxin

Five male and five female mice received a single dose of 3,280 mg/kg of CryIA(b) δ-endotoxin by oral gavage. No animals died nor were there significant clinical signs as a result of the exposure. One female failed to gain weight between day 7 and day 14. All animals gained weight by the end of the study. Males gained more weight over the study than females. The LD_{50} was therefore greater than 3280 mg/kg, the highest dose tested.

2. In-Vitro Digestibility of CryIA(b) δ-endotoxin

The CryIA(b) δ-endotoxin from either corn or *Bacillus thuringiensis kurstaki* HD1-9 is rapidly degraded in the presence of pepsin. Using 1/1000 strength pepsin, a time course study shows that the introduced δ-endotoxin from either source degrades within 10 minutes to fragments that lack any immunorecognition in a western blot assay. While this study provides useful information demonstrating the digestibility of the CryIA(b) δ-endotoxin produced in corn, it is not yet a validated study for assessing protein toxicology. It is not clear whether lack of toxicity correlates with *in vitro* digestibility under the conditions of the assay. EPA was relying on this study to demonstrate rapid degradation of the δ-endotoxin.

3.Acute Oral Toxicity of Corn Leaf Protein Extracted from *Bt* Corn

Application of this study to dietary risk assessment is not possible because of extremely low doses administered, small test populations and unexplained deaths occurring in both control and treated groups. Therefore, EPA is not relying on this study to support the tolerance exemption.

RESIDUE CHEMISTRY DATA

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(b) δ-endotoxin was shown to have an LD_{50} greater than 3280 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(b) δ-endotoxin is not considered acutely or chronicly 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 genetic material necessary for the production of the *Bacillus thuringiensis* CryIA(b) δ-endotoxin are the nucleic acids (DNA) which comprise: (1) genetic material encoding the CryIA(b) δ-endotoxin and (2) its regulatory regions. "Regulatory regions" are the genetic material that control the expression of the genetic material encoding the CryIA(b) 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(b) δ-endotoxin in corn. 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(b) δ-endotoxin and the genetic material necessary for its production (plasmid vector pCIB4431) in corn.

TOLERANCE EXEMPTION

Based on the information considered, we have concluded that establishment of a tolerance is not necessary to protect the public health. This exemption from the requirement of a tolerance eliminates the need to establish a maximum permissible level for residues of this plant-pesticide in all raw agricultural commodities of field corn, sweet corn, and popcorn.

2. PAT

TOXICOLOGY ASSESSMENT

EPA evaluated an acute oral toxicity study and an *in vitro* digestibility study. In the acute mouse oral toxicity study, a 51% PAT protein mixture was shown to have an LD_{50} greater than 5050 mg/kg. The Agency also expects that enzymes with no

significant amino acid homology to known 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 PAT enzyme meets all the above criteria, and as predicted, submitted data show that no toxicity results when high doses of this protein are administered orally to laboratory rodents. 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 PAT protein is not considered acutely or chronicly toxic. The PAT acute oral toxicity study together with data indicating that the PAT protein is rapidly degraded in the gastric environment and is also readily denatured by heat or low pH are sufficient to support a finding of no unreasonable acute mammalian oral toxicity for the PAT protein.

The genetic material necessary for the production of the PAT protein are the nucleic acids (DNA) which comprise the (1) genetic material encoding the phosphinothricin acetyltransferase and (2) its regulatory regions. "Regulatory regions" are the genetic material that control the expression of the genetic material encoding the phosphinothricin acetyltransferase, 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 inert 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 PAT protein in corn.

Allergenicity -

Current scientific knowledge suggests that common food allergens tend to be resistant to degradation by heat, acid, and proteases, are glycosylated and are present at high concentrations in the food. Ciba Seeds has submitted data which indicates the PAT protein is rapidly degraded in the gastric environment and is also readily denatured by heat or low pH.

Submitted Data-

1. Acute Oral Toxicity of Bacterially Produced PAT Protein

A white powder (PAT-0195) containing 51% PAT enzyme by

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weight was obtained by purification from an *E. coli* fermentation and dosed at 5050 mg/kg to mice. No treatment related significant toxic effects were seen 14 days after oral gavage of high levels of the purified PAT marker protein.

2. *In-Vitro* Digestibility of PAT Protein

The 22,000 M.W. PAT enzyme is rapidly degraded in the presence of pepsin or low pH so that it loses enzymatic activity and is not detected by SDS-PAGE. The enzyme also loses activity if subject to temperatures over 35°C. EPA was relying on this study to demonstrate rapid degradation of the protein.

3. Acute Oral Toxicity of Corn Leaf Protein Extracted from *Bt*/PAT Corn

Application of this study to dietary risk assessment is not possible because of extremely low doses administered, small test populations, and the unexplained deaths occurring in both control and treated groups. Therefore, EPA is not relying on this study to support the tolerance exemption.

RESIDUE CHEMISTRY DATA

Residue chemistry data were not required because of the lack of mammalian toxicity of this active ingredient. 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 PAT protein is not considered acutely or chronicly toxic. This is similar to the Agency position regarding toxicity and the requirement of residue data for the microbial *Bacillus thuringiensis* products. [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 genetic material necessary for the production of the PAT protein are the nucleic acids (DNA) which comprise (1) genetic material encoding the phosphinothricin acetyltransferase and (2) its regulatory regions. "Regulatory regions" are the genetic material that control the expression of the genetic material encoding the phosphinothricin acetyltransferase, 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 PAT protein corn. Therefore, no residue data are required in order to grant an exemption from the requirement of a tolerance for the plant-

pesticide inert ingredient: phosphinothricin acetyltransferase (PAT) and the genetic material necessary for its production (plasmid vector pCIB3064) in corn.

TOLERANCE EXEMPTION

Based on the information considered, the Agency concludes that establishment of a tolerance is not necessary to protect the public health. This exemption from the requirement of a tolerance eliminates the need to establish a maximum permissible level for residues of this plant-pesticide inert ingredient in all raw agricultural commodities of field corn, sweet corn, and popcorn.

B. ENVIRONMENTAL FATE

Based on data submitted by Ciba Seeds, it appears that the expression of the CryIA(b) δ-endotoxin will occur at the highest levels in leaves, pollen, and whole plants. The highest levels detected in any individual samples were 4.4, 7.1, and 0.6 μg/g fresh weight for leaves, pollen, and whole plants, respectively. CryIA(b) levels were below the level of detection (5 ng/g fresh weight) for seed, and at trace levels (⟨8 ng/g fresh weight) for pith and roots. Generally the levels were highest in seedlings, and declined through anthesis and seed maturity, and reached the lowest levels at senescence. There was no detectable levels in silage. Commercial plantings of the corn expressing the *Bt* plant-pesticide would result in expression of the cryIA(b) gene in the seed of other corn plants. Corn is a wind-pollinated species, and separate tassel and silk flowers encourage natural outcrossing within and between fields of corn. Although expression of the CryIA(b) δ-endotoxin would confer a degree of insect resistance to the transformed plants, there are other traits which preclude any significant risk of *Bt* maize plants becoming weeds as a result of the expression of the toxin. Cultivated maize has been bred for survival under cultivation only, and is limited in its ability to proliferate as a weed or to survive in the wild by such traits as a non-shattering seed habit, lack of seed dormancy, and lack of cold hardiness.

There is no significant risk of gene capture and expression of the *Bt* plant-pesticide by wild or weedy relatives of corn in the United States, its possessions or territories because there are no sexually compatible wild or weedy relatives of corn (*Zea mays*) in the United States, its possessions or territories.

C. ECOLOGICAL EFFECTS

A review of the studies submitted for the registration of the *B. thuringiensis* CryIA(b) produced in corn demonstrated a lack of adverse effects to birds, aquatic invertebrates, honey bee larvae, coccinellid predators, and earthworms. However, it was toxic to a collembolan species (*Folsomia candida*), which was selected as one of the soil invertebrate test organisms. If senescent post-harvest corn plants containing the CryIA(b) δ-endotoxin were tilled into the top 6 inches of soil a maximum of 4.2×10^{-4} mg toxin/kg of soil would be present. If a deeper disking or plowing depth were used then the concentration of toxin would be less. The NOEC level for the collembolan test species is 8.8×10^{-2} mg toxin/kg of soil, thus a safety factor of approximately 200-fold exists. In addition, one of the one of the reasons for requiring the nontarget soil invertebrate tests (earthworm and Collembola) was the concern that adverse effects on the collebolan species would cause a build up of plant detritus in corn fields. However, the long term soil use of highly toxic chemical insecticides, such as phorate and carbofuran which probably have adverse impacts on that species, has not resulted in the build-up of plant detritus in corn fields. Thus, an observable deleterious effect on the soil ecosystem is not expected to result from the growing of CryIA(b) δ-endotoxin-containing corn plants.

Avian Studies

These results indicated that the *Bt* CryIA(b) δ-endotoxin produced in corn is practically non-toxic to bobwhite quail on an acute basis. When administered by oral gavage at a dosage of 2,000 mg protein/kg body weight, *Bt*-corn had no apparent effect upon bobwhite quail after 14 days. The acute toxicity LD_{50} value to quail was determined to be greater than 2,000 mg protein/kg body weight. In view of the lack of acute toxicity with *Bt*-corn, no avian hazard is expected from the proposed uses of this plant-pesticide.

The requirement for an avian dietary test will be waived based on information supplied by the registrant which supports the claims of a lack of toxicity in acute avian testing, the non-persistent and non-bioaccumulative characteristics of CryIA(b) protein, and the low potential for chronic exposure of birds to significant concentrations of CryIA(b).

Fish Studies

The requirement for a static renewal toxicity study has been waived based on a lack of exposure of fish to the *Bt* endotoxin CryIA(b) protein produced in corn. Ciba Seeds determined through bioassay testing using the ECB (), that a sample fish diet made using corn containing the *Bt* δ-endotoxin did not adversely affect ECB larvae and an analysis using ELISA indicated that CryIA(b)

was not detectable in the feed samples. Therefore, fish eating a food mix made from corn containing the *Bt* δ-endotoxin would not be exposed to detectable active *Bt* δ-endotoxin protein.

Mammalian Wildlife

These studies are required only when toxicology data are inadequate for assessment of hazard to wild mammals. The data submitted to EPA indicate that there is no significant toxicity to rodents from acute oral testing at the maximum hazard dose. Since the anticipated exposure of mammalian wildlife is considered high, risk to wild mammals from the *Bt* CryIA(b) endotoxin is a potential concern. However, in light of the above toxicology information, risk to mammalian wildlife is expected to be minimal to nonexistent.

Aquatic Invertebrate Studies

A 48-hour static renewal toxicity study of maize pollen containing *Bt* CryIA(b) δ-endotoxin was conducted using *Daphnia magna*. Test daphnids were dosed at five concentration levels, including a maximum hazard dose of 150 mg/L (nominal) of water. No mortalities were observed at any of the treatment levels tested. The 48-hour EC_{50} was determined to be greater than 150 mg/L. The LOEC (lowest observed effect concentration) and NOEC (no observed effect concentration) were found to be 150 mg/L.

These results indicate that *Bt* CryIA(b) δ-endotoxin expressed in corn is practically non-toxic to *Daphnia magna*. In view of the above results, no freshwater aquatic invertebrate hazard is expected from the use of this product.

Estuarine and Marine Animal Studies

The Estuarine fish study was not required for this product because of very low potential for exposure.

Nontarget Plant Studies

Since the a.i. in this product is an insect toxin (*Bt* endotoxin) that has never shown any toxicity and/or pathogenicity to plants, these studies have been waived for this product.

Honey Bee Studies

The study was scientifically sound and demonstrated no significant treatment effects on honey bee larvae from *Bt* endotoxin in corn pollen.

Nontarget Beneficial Organism Studies

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1. Lady beetle predator: This study was scientifically sound and demonstrates that corn pollen containing the CryIA(b) toxin should not cause significant adverse effects to lady beetle predators. The study is judged to be supplemental and does not need to be repeated.

2. Earthworm: This study was scientifically sound and demonstrates that the CryIA(b) protein toxin is practically nontoxic to earthworms.

3. Collembola: This study was scientifically sound and demonstrates that the CryIA(b) protein, when added to an artificial soil mix, caused significant mortality to Collembola and significantly reduced reproduction of the survivors. The LD_{50} was 240 mg *Bt* maize leaf protein/kg of soil (95% CL 210-280). The LOEL (lowest observed effect level) was 250 mg/kg, and the NOEL (no observed effect level) was 125 mg/kg. The MATC (maximum acceptable toxicant concentration) was between 125 mg/kg and 250 mg/kg, with geometric mean of 180 mg/kg.

Nontarget Insect Study

This study was not specifically required for the registration of this product. It was submitted voluntarily by the registrant to determine whether *Bt* plant-pesticide maize, expressing truncated *Bt*-derived CryIA(b) δ-endotoxin, had a significant negative impact on natural nontarget insect populations. The study did provide some useful information on the differences in toxicity to nontarget insect species between chemical insecticides and *Bt*-expressing corn plants, but had some deficiencies. Therefore, the study will be considered supplemental. Since this was not a required study, it will not have to be repeated.

Impact of Resistance on Beneficial Insects

See section III.A.2 for a complete discussion.

Endangered Species Considerations

A Biological Opinion 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 corn plants, EPA believes that the Biological Opinion is inapplicable, and that reinitiation of consultation is not required.

The primary route of exposure from foliar *Bt* sprays is

through either direct application or as a result of spray drift.

Conversely, the primary route of exposure to the *Bt* toxin expressed in corn plants is through ingestion of corn tissue. There are no reports of endangered/threatened species feeding on corn plants, thus corn tissue containing the CryIA(b) δ-endotoxin would not be consumed by such species. Corn is widely grown, and above ground feeding damage is easily observed on corn plants due to its morphology and the way it is grown. Consequently, the identities of organisms that feed on corn are well established. In addition, because there are no sexually compatible wild or weedy relatives of corn (*Z. mays*) in the United States, its possessions or territories, the CryIA(b) δ-endotoxin gene cannot escape into plants on which endangered/threatened species feed on in these areas.

Although corn pollen containing the CryIA(b) δ-endotoxin can drift out of corn 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 corn plants containing the cryIA(b) gene are grown. The amount of pollen that would drift from a these corn 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(b) δ-endotoxin.

Because EPA expects that in most cases, no listed endangered species of Lepidoptera will be exposed to the *Bt* protein expressed in corn 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.

D. RESISTANCE MANAGEMENT

1) Resistance Management Assessment

Ciba Seeds and Mycogen Plant Sciences have developed a resistance management plan for the CryIA(b) δ-endotoxin produced in corn. After reviewing all of the available information on the potential for ECB resistance to develop to the CryIA(b) protein produced in corn, the Office of Pesticide Program's Pesticide Resistance Management Workgroup (PRMW) concluded that Ciba Seeds and Mycogen Plant Sciences have a workable basis for a resistance management plan that would reduce the possibility of resistance developing for 3 to 5 years following the introduction of seed corn producing cryI δ-endotoxins. The components of Ciba Seeds' and Mycogen's resistance management plans include: knowledge of pest biology, high dose expression strategy, refugia, monitoring, development of non-target pest resistance, development of cross-resistance, employment of IPM, education/communication strategies, and development of alternative modes of action.

Although, no resistant ECB populations have been detected in the field, resistant ECB populations have been selected for in the laboratory using microbial *Bt* formulations. These results indicate that ECB have the genetic potential to develop resistance to the CryIA(b) δ-endotoxin.

The PRMW concluded that there is a potential for resistance to develop to other δ-endotoxins of the CryI class. The potential for cross-resistance to members of the cryI class has been demonstrated for diamondback moth and tobacco budworm in a number of laboratory-selected insect populations using foliar *Bt* biopesticides as selective agents.

The PRMW concluded that there is a potential for corn earworm (CEW) (*Helicoverpa zea* (Boddie)) resistance developing in both corn and cotton. Some Lepidoptera which primarily feed on corn have alternative hosts, such as cotton. The threat of cross-resistance or multiple resistance between the various CryI (also CryIIA) δ-endotoxins exists for the ECB, CEW, and possibly other Lepidoptera on corn.

The PRMW concluded that cross-resistance and/or multiple resistance to many different Cry I δ-endotoxins could lead not only to the loss of the CryIA(b) δ-endotoxin produced in Event 176-derived corn hybrids, but other *Bt* microbial pesticides. CryIA(b) δ-endotoxins are part of many different *Bt* microbial pesticide formulations used on a wide range of crops and ornamentals. If CEW develops resistance to the CryIA(b) δ-endotoxin produced in Event 176-derived corn hybrids, it could pose a problem in cotton in which *Bt* microbial pesticides are used. In addition, the development of cross resistance could lead to problems controlling CEW/bollworm using either transgenic cotton producing *Bt* δ-endotoxins or *Bt* microbial pesticides containing the CryIA(b) δ-endotoxin or δ-endotoxins other than CryIA(b). CEW/bollworm resistance could lead to the failure of *Bt* microbial pesticide formulations and the loss of *Bt* microbial pesticides as resistance management option for ECB and other insects.

The PRMW concluded that Ciba Seeds' and Mycogen Plant Science's resistance management plans should adequately manage ECB resistance to the cryI δ-endotoxins produced in corn in the first 3 to 5 years following commercialization because it is unlikely that these hybrids will dominate the market during this period. As long as all commercial seed corn production is not immediately switched, on a local or national level, to corn producing CryI δ-endotoxins, the "unstructured, market-driven refugia" described by Ciba Seeds and Mycogen Plant Sciences should be adequate for approximately 3 to 5 years following initial commercialization because there will be natural refugia between fields (spatial mosaics). Because of the larval mobility and availability of alternate hosts, seed mixes will not be

viable refugia. Therefore, "structured" refugia, where there are blocks of *Bt* plant-pesticide corn and conventional corn or other host plants, are necessary.

Market projections provided by Ciba Seeds and Mycogen Plant Sciences, and other information available to the PRMW indicate that such unstructured refugia should exist during this initial commercialization period. However, the PRMW notes that after this initial period of CryI corn introduction, "structured" refugia will be necessary to ensure the success of the proposed resistance management strategy. The resistance management strategy will likely be unworkable after three to five years following initial commercialization without structured refugia.

Ciba Seed and Mycogen Plant Sciences have chosen a high dose expression strategy to employ for their corn hybrids producing the CryIA(b) δ-endotoxin. The PRMW believes that the laboratory bioassay data and field data presented by Ciba Seeds and Mycogen Plant Sciences provide preliminary evidence in support of the high dose expression strategy. However, the evidence for the high dose expression strategy is limited and is not necessarily representative of the selective conditions operating in the field. The high dose expression strategy will need to be validated in the field to determine whether the CryIA(b) δ-endotoxin will be produced uniformly at high enough doses in the field to kill all susceptible individuals. Without field validation, it is impossible to predict at this time the absolute success of the high dose expression strategy.

The major deficiency in Ciba Seeds' and Mycogen Plant Sciences's resistance management strategy is in the area of what will be required for implementation of a "structured" refugia. The unanswered question is how to structure refuges and how large a refuge is needed after the first several years of commercialization, when the acreage devoted to *Bt* plant-pesticide corn producing the CryIA(b) or CryIA(c) δ-endotoxins will be of a significant size to warrant more structured refugia. There is no agreement among research scientists and industry on refugia size requirements. Various research entomologists have provided their thoughts on this subject to the PRMW. The values for adequate refuge size primarily range from 20% to 50% of non-*Bt* plant-pesticide corn or other host plants per farm.

Therefore, the PRMW believes that the minimum adequate size or structure for a "structured" refuge cannot be determined until further research is conducted. The PRMW recommends that Ciba Seeds and Mycogen Plant Sciences continue their research efforts to optimize the "structural" refugia strategy and educational efforts to work with growers on using an appropriate resistance management strategy. The success of the high dose strategy, the mitigation of resistance, and inevitably, the longevity of the Event 176-derived hybrids or other corn hybrids producing CryI

proteins, is dependent on the appropriate use of "structured" refuges. Once these data are collected, the PRMW can determine the "appropriate" minimum non-plant-pesticide refugia size.

2) Resistance Management Risk Mitigation

BPPD is recommending that we require that Ciba Seeds and Mycogen Plant Science do the following as terms and conditions of this new use in order to mitigate the resistance management risks outlined above and in the section III of this document.

1. The registrations will automatically expire on midnight August 2, 2000. EPA will reevaluate the effectiveness of Ciba Seeds' and Mycogen Plant Science's resistance management plans before August 2, 2000 and decide whether to convert the registration to a non-expiring registration.

2. The amendments to the existing FIFRA section 3(c)(5) registrations are for field corn only. The terms and conditions for sweet corn and popcorn in Ciba Seeds' and Mycogen Plant Science's FIFRA section 3(c)(5) registration remain in effect.

3. Ciba Seeds and Mycogen Plant Sciences will:

a. unless demonstrated to EPA's satisfaction that alternative resistance management practices are equally or more effective than a structured refugia, develop a plan for "structured" refugia within three years from the date of the amended conditional registration;

b. discuss the development and implementation of the plan and alternative resistance management practices with EPA throughout development and implementation; and

c. implement an EPA approved "structured" refugia plan or an EPA approved alternative resistance management plan no later than August 2, 2000.

4. Ciba Seeds and Mycogen Plant Sciences will monitor for the development of resistance using baseline susceptibility data and/or a discriminating concentration assay when such an assay is available. Ciba Seeds and Mycogen Plant Sciences will continue efforts to develop a discriminating concentration assay. Ciba Seeds and Mycogen Plant Sciences will ensure that monitoring studies are conducted annually to determine the susceptibility of ECB populations to the CryIA(b) protein. Populations of ECB will be collected from representative distribution areas of the registrant's Event 176-derived hybrids, with particular focus on those areas of highest distribution. The results of such monitoring studies will be communicated to the Agency on an annual basis, by January 31 of the year following the population collections for a given growing season.

In addition, Ciba Seeds and Mycogen Plant Sciences will instruct their customers (growers and seed distributors) to contact Ciba Seeds and Mycogen Plant Sciences (e.g., via a toll-free customer service number) if incidents of unexpected levels of ECB damage occur. Ciba Seeds and Mycogen Plant Sciences will investigate and identify the cause for this damage by local field sampling of plant tissue from their hybrids and sampling of ECB populations, followed by appropriate *in vitro* and *in planta* assays. Upon Ciba Seeds and Mycogen Plant Sciences' confirmation by immunoassay that the plants contain CryIA(b) protein, bioassays will be conducted to determine whether the collected ECB population exhibits a resistant phenotype.

Until such time that a discriminating concentration assay is established and validated, Ciba Seeds and Mycogen Plant Sciences will utilize the following to define a confirmed instance of ECB resistance:

Progeny from the sampled ECB population will exhibit both of the following characteristics in bioassays initiated with neonates:

a. An LC50 in a standard CryIA(b) diet bioassay that exceeds the upper limit of the 95% confidence interval of the mean historical LC50 for susceptible ECB populations, as established by the ongoing baseline monitoring program. The source of CryIA(b) crystal protein standard for this bioassay will be *Bacillus thuringiensis* subsp. *kurstaki* strain HD1-9.

b. > 30% survival and > 25% leaf area damaged in a 5-day bioassay using CryIA(b)-positive leaf tissue under controlled laboratory conditions.

Based upon continued experience and research, this working definition of confirmed resistance may warrant further refinement. In the event that Ciba Seeds and Mycogen Plant Sciences find it appropriate to alter the criteria specified in the working definition, Ciba Seeds and/or Mycogen Plant Sciences must obtain Agency approval in establishing a more suitable definition.

5. Ciba Seeds and Mycogen Plant Sciences will report all instances of confirmed ECB resistance, as defined above, to the Agency within 30 days. Upon identification of a confirmed instance of ECB resistance Ciba Seeds and Mycogen Plant Sciences will take the following immediate mitigation measures:

a. notify customers and extension agents in the affected area,

b. recommend to customers and extension agents in the

affected area the use of alternative control measures to reduce or control the local ECB population, and

c. recommend to customers and extension agents in the affected area that crop residues be incorporated into the soil following harvest, to minimize the possibility of overwintering of ECB.

Within 90 days of a confirmed instance of ECB resistance, as defined above, Ciba Seeds and Mycogen Plant Sciences will: (1) notify the Agency of the immediate mitigation measures that were implemented, and (2) submit to the Agency a proposed long-term resistance management action plan for the affected area, (3) work closely with the Agency in assuring that an appropriate long-term resistance management action plan for the affected area is implemented, (4) and implement an action plan that is approved by EPA and that consists of some or all the following elements:

a. Informing customers and extension agents in the affected area of ECB resistance,

b. Increasing monitoring in the affected area, and ensuring that local ECB populations are sampled on an annual basis,

c. Recommending alternative measures to reduce or control ECB populations in the affected area,

d. Implementing a structured refuge strategy in the affected area based on the latest research results. The implementation of such a strategy will be coordinated by the Agency with other registrants.

e. If the above elements are not effective in mitigating resistance, Ciba Seeds and Mycogen Plant Sciences will voluntarily cease sale of all of Ciba Seeds and Mycogen Plant Sciences' CryIA(b) corn in the county experiencing loss of product efficacy to this active ingredient and the bordering counties until an effective local management plan approved by EPA has been implemented. During the voluntary suspension period, Ciba Seeds and Mycogen Plant Sciences may sell and distribute in these counties only by obtaining EPA approval to study resistance management in those counties. The implementation of such a strategy will be coordinated by the Agency with other registrants.

If EPA agrees that an effective resistance management plan has been implemented which mitigates resistance, Ciba Seeds and Mycogen Plant Sciences can resume sales in the affected county(ies).

6. Ciba Seeds and Mycogen Plant Sciences will maintain a database to track its sales (units and location) of Event 176-derived corn on a county-by-county basis, to the extent that such data are available. Ciba Seeds and Mycogen Plant Sciences will provide annually sales data for each state indicating the number of units of Event 176-derived hybrids that it sells. This information will be provided by January 31 of the year following each growing season.

7. Ciba Seeds and Mycogen Plant Sciences will provide grower education. Ciba Seeds and Mycogen Plant Sciences will include an active partnership with such parties as: university extension entomologists and agronomists, consultants, and corn grower groups. Ciba Seeds and Mycogen Plant Sciences will implement a grower education program directed at increasing grower awareness of resistance management, in order to promote responsible product use. As specific resistance management recommendations are developed (e.g., as a result of ongoing research or experience) these will be incorporated, as appropriate, into the various grower communication and educational media. Ciba Seeds and Mycogen Plant Sciences will inform the Agency as they develop, implement, and refine their communication strategies. In addition to grower communication vehicles, Ciba Seeds and Mycogen Plant Sciences will also develop a Grower Guide, to be distributed to all customers, that will include current information regarding resistance management and integrated pest management.

8. Ciba Seeds and Mycogen Plant Sciences will each develop a resistance management program that is acceptable to EPA and that includes the research specified in paragraph 8, a. through e., of this section. They will each confer with EPA as they develop various aspects of that program and will submit annual progress reports to EPA including results and conclusions from research and the scientific literature as they became available in the research areas listed in paragraph 8, a. through e. of this section. They will each conduct the research specified in paragraph 8, a. through e., and any other research that is included in the program that EPA accepts. They will each use the research as a basis to develop a long-term resistance management strategy.

a. ECB pest biology and behavior including adult movement and mating patterns, larval movement, survival on silks, kernels, and stalks, and overwintering survival and fecundity on non-corn hosts.

b. The feasibility of "structured" refuge options for ECB including "block" refugia, "50-50 early/late season patchwork", and other possibilities.

c. Development of a discriminating concentration

(diagnostic concentration) assay for field resistance (field screening) for ECB.

d. Effects of corn producing the CryIA(b) δ-endotoxin on pests other than ECB, such as corn earworm, and the stalk borer complex.

e. The biology of ECB resistance including receptor-mediated resistance and its potential effect on population fitness, as well as the effects on insect susceptibility to other Cry proteins.

V. POTENTIAL BENEFITS

BIOLOGICAL ANALYSIS

The ECB is found throughout much of the United States where corn is grown, including the corn belt of the upper Midwest. The ECB does cause variable damage to the corn crop on a seasonal basis. Losses can reach a 10 to 20 percent yield loss. Growers usually either do not find conventional control methods profitable or do not recognize the seriousness of the problem. As a result, most do not apply foliar controls.

The Ciba Seeds *Bt* corn plant-pesticide, which was developed to control the ECB (*Ostrinia nubilalis* (Huebner)), contains the gene CryIA(b), event 176 from *Bacillus thuringiensis kurstaki*. The gene is expressed in corn leaves and pollen, but not in the silk and kernels. The active ingredient in the corn plant does not begin to break down until the corn plant begins to senesce. At this time the plant is not particularly attractive to ovipositing ECB females.

Pest Spectrum

Efficacy data show the ECB will be controlled by this *Bt* plant-pesticide. There is a likelihood that there also will be control of other Lepidoptera pests of corn, such as cutworms, corn earworm, armyworm, fall armyworm, and several corn stalk borers. However, no data were submitted to show the degree of efficacy. Non-Lepidoptera, including beneficial species, are less likely to be impacted by *Bt* plant-pesticides in corn than by the broad spectrum insecticides normally used.

Suitability for IPM Programs

Utilization of *Bt* plant-pesticides in corn to control the ECB would fit in with IPM programs, now and in the foreseeable future. It will not preclude use of conventional insecticides or other pesticides for other corn pests such as chinch bugs, cornleaf aphid, grasshoppers or the corn rootworm complex. It is compatible with any nonchemical or reduced chemical program.

ECONOMIC ANALYSIS

Adoption of *Bt* plant-pesticides in corn is expected to increase yield to some corn growers with ECB problems as much as 10 to 20 percent. While exact distribution of ECB problems was not available to the Agency, there were adequate information to indicate that a substantial portion of corn growers would find it profitable to shift to corn which produces the *Bt* protein if the seed producers price the seed correctly. Although it is not possible to quantify the monetary benefits growers would obtain through the use of *Bt* plant-pesticides in field corn, 10 to 20 percent is a significant yield increase for any user of this product.

Information available to the Agency indicates that substantial benefits may be available to corn producers from the use of *Bt* plant-pesticides in corn. The registrants are likely to capture some portion of these benefits in terms of higher price for the seed with the remainder going to farmers and consumers of corn.

Effect on Current Pesticide Usage

Currently, relatively little pesticide is applied to field corn for control of these pests. Available information indicates less than 5 percent of field corn is treated with a foliar insecticide in a typical year to control the entire range of foliar insect pests. *Bt* plant-pesticides in corn could essentially eliminate that portion which was for pests controlled by this technology and may reduce the use of other insecticides where beneficial insects currently killed by chemical insecticides used to control ECB are now able to survive and control other corn insect pests.

VI. 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). Ciba has submitted and Mycogen has cited

satisfactory data pertaining to the proposed additional use of the products in field corn, 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 enough data to characterize the incremental risks associated with the development of resistance resulting from approval of their applications.

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 is 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 IV, D above, the introduction of these products for any wide-scale use poses the risk that pests, such as the ECB and CEW, 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 3 to 5 years following initial commercial use of these products. Additional data, however, is 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 IV, D, 2, para. 8, a-e.

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 addition use does not "significantly increase the risk of any unreasonable adverse effect." In essence, FIFRA requires a determination that the proposed additional use of these products for food and feed 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 these products for food and feed poses the risk of the development of multiple- and cross-resistance in certain Lepidoptera pests on corn. As a result pests could develop resistance to certain microbial *Bt* pesticides that are applied to both field corn 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 pesticide alternatives. The Agency further recognizes that microbial *Bt* pesticides have low dietary, worker, and ecological risks when compared to the more hazardous alternatives that might

replace the microbial *Bt* pesticides should resistance develop. The microbial 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.

These registrations may provide substantial benefits to corn producers in the form of increased yields of field corn resulting from the control of damage caused by the ECB and possibly other corn pests. The registrants also may benefit from the higher sales price for seed that contains the product.

The risks are substantial and BPPD has concluded that the risks, if unchecked, would outweigh the benefits of the proposed new use. However, the terms and conditions of registration that are recommended in Section IV, D, 2 of this Decision Document would mitigate the risks from pesticide resistance sufficiently so that the risks of the proposed registrations would not significantly increase the risks of unreasonable adverse effects. Both registrations will expire automatically in August, 2000. At that time EPA can re-evaluate whether each registrant has an effective resistance management plan. In the interim, each registrant must conduct a grower education program directed at increasing grower awareness of resistance management; conduct monitoring to help detect the development of resistance in ECB to the CryIA(b) δ-endotoxin and stop selling the product in areas where the registrant has detected resistance to its own product; conduct research to determine how to develop an effective long-term resistance management plan; and implement an EPA-approved structured refugia system.

B. EXISTING FIFRA 3(c)(5) REGISTRATION

Ciba and Mycogen have existing registrations granted pursuant to FIFRA section 3(c)(5) that authorize the use of the plant-pesticide in the propagation of seed for any type of corn. BPPD recommends leaving those registrations in place for the propagation of seed for sweet corn and popcorn.

The existing seed propagation registrations for all types of corn are subject to a variety of terms and conditions that limit the scope and duration of the use. The limited scope and duration of those registrations allowed the Agency to determine that the pesticides could be registered under FIFRA section 3(c)(5) even though the science data review was not complete, the tolerance exemption petition was still under review, and the SAP report had not been analyzed completely.

Those steps are now complete except for the scientific analysis of the potential risks associated with the development of resistance to the pesticides during full-scale commercial production of sweet corn and popcorn for food and feed use.

Because the resistance analysis is not complete, BPPD recommends leaving in place the existing FIFRA 3(c)(5) registration for the propagation of sweet and pop corn seed under the current terms and conditions of registration. The existing terms and conditions impose limitations that would continue to insure that resistance does not develop during the propagation of seeds for sweet corn and popcorn.

VII. 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. 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 Ciba and Mycogen's plant-pesticide products containing the active ingredient *Bacillus thuringiensis* CryIA(b) δ-endotoxin and the genetic material necessary for its production in field corn be CONDITIONALLY REGISTERED for food and feed use under 3(c)(7)(B) of FIFRA.

CONCUR: Penelope A Fenner-Crisp

NONCONCUR: _____

DATE: 8/8/95