Office of Pesticide Programs

Biopesticide Fact Sheet

Bacillus thuringiensis Cry1Ab Delta-Endotoxin and the Genetic Material Necessary for Its Production (Plasmid Vector pCIB4431) in Corn [Event 176] (006458)

Issued: 4/00

Fact Sheet	Technical Doc	Products	Registrants	Regulatory Activity	FR Notices	Bibliography

Reason for Issuance: Update to Include New Requirements for the 2000 Growing Season, Details of the Non-Target Butterfly Data-Call-In; and Updated Gene Flow, Insect Resistance Management, and Ecological Effects Sections

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I. DESCRIPTION OF THE PLANT PESTICIDE

Bacillus thuringiensis Cry1Ab Delta-Endotoxin and the Genetic Material Necessary for Its Production (Plasmid Vector pCIB4431) in Corn

OPP Chemical Code: 006458

Year of Initial Registration: 1995

Pesticide Type: Insecticide

U.S. and Foreign Producers:

Novartis Seeds, Inc. - Field Crops - NAFTA
P.O. Box 12257
Research Triangle Park, NC 27709-2257

Mycogen Seeds
c/o Dow Agrosciences, LLC
9330 Zionsville Road
Indianapolis, IN 46268-1054

Note: *Ciba Seeds became part of Novartis Seeds subsequent to the formation of Novartis from Sandoz and Ciba Geigy.*

II. USE SITES

Commercial Use in Field Corn and Popcorn.

The registrations are conditional upon data development in the area of resistance management research and limited by resistance management and expiration provisions of the registration.

III. SCIENCE FINDINGS

The Agency believes that the products will perform their intended function and can foresee no unreasonable adverse effects to humans, nontarget organisms, or the environment from the use of these products.

A. HUMAN HEALTH

1. 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* Cry1Ab delta-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 Cry1Ab delta-endotoxin in corn, the acute oral toxicity, and *in vitro* digestibility of the delta-endotoxin.

a. 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 Cry1Ab 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 [Sjoblad, 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 Cry1Ab delta-endotoxin is not considered acutely or chronically toxic. Adequate information was submitted to show that the test material derived from microbial cultures was biochemically and insecticidally similar to the delta-endotoxin as produced by corn. Microbial production of Cry1Ab delta-endotoxin was chosen in order to obtain sufficient material for mammalian testing. In addition, the *in vitro* digestibility studies indicate the delta-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* Cry1Ab delta-endotoxin are the nucleic acids (DNA) which comprise (1) genetic material encoding the Cry1Ab delta-endotoxin and (2) its regulatory regions. "Regulatory regions" are the genetic material that control the expression of the genetic material encoding the Cry1Ab delta-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* Cry1Ab delta-endotoxin in corn.

b. 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 Cry1Ab delta-endotoxin is rapidly degraded by gastric fluid *in vitro*, is not present as a major component of food (*i.e.*, is present in only trace quantities in corn kernels and is not detectable in finished silage or processed corn products) 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 delta-endotoxin in the crystal protein. Recent *in vitro* studies also confirm that the delta-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 delta-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.

2. Residue Chemistry Data

Residue chemistry data were not required because of the lack of mammalian toxicity of this active ingredient. In the acute mouse oral toxicity study, the Cry1Ab delta-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 [Sjoblad, 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 Cry1Ab delta-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 genetic material necessary for the production of the *Bacillus thuringiensis* Cry1Ab delta-endotoxin are the nucleic acids (DNA) which comprise: (1) genetic material encoding the Cry1Ab delta-endotoxin and (2) its regulatory regions. "Regulatory regions" are the genetic material that control the expression of the genetic material encoding the Cry1Ab delta-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* Cry1Ab delta-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* Cry1Ab delta-endotoxin and the genetic material necessary for its production (plasmid vector pCIB4431) in corn.

Tolerance Exemption

Based on the information considered, the Agency concluded that establishment of a tolerance is not necessary to protect the public health. Therefore an exemption from the requirement of a tolerance has been established which 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

B. ENVIRONMENTAL FATE AND GENE FLOW

Based on data submitted by Ciba Seeds, it appears that the expression of the Cry1Ab delta-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. Cry1Ab levels were below the level of quantification for seed (5 ng/g fresh weight) and for pith and roots (8 ng/g fresh weight). Generally the levels were highest in seedlings, and declined through anthesis and seed maturity, and reached the lowest levels at senescence. There was none detectable in silage.

EPA has reviewed the potential for gene capture and expression of the Cry1Ab, Cry1Ac, and Cry9C endotoxin genes from *Bt* plant-pesticides, as expressed in corn plants, by wild or weedy relatives of maize in the United States, its possessions and territories. Following this review, EPA believes there is no significant risk of gene capture and expression of any of the Cry endotoxins by wild or weedy relatives of maize in the United States because extant populations of sexually compatible species related to *Zea mays* (maize or corn) are not present in the United States or its territories and possessions.

Zea mays is a wind-pollinated species, and the presence of spatially separate tassels (male flowers) and silks (female flowers) encourages outcrossing among nearby plants. Maize cultivars and landraces are known to be interfertile to a large degree. Recent studies have indicated that cross-pollination at 100 ft from the source of genetically modified maize was 1 % and this proportion declined exponentially to 0.1 % at 130 ft and further declined to 0.03 % at the farthest distance measured (160 ft). For production of Foundation Seed, a distance of 660 ft has been required to ensure separation of pollen types. Additionally, the relatively large size of corn pollen as compared to other grass species and the short time span that corn pollen remains viable (i.e., typically less than 60 minutes) under natural conditions both preclude long distance transfer for purposes of outcrossing. Under conditions of high temperature and desiccation, corn pollen longevity is measured in minutes. These conditions may even destroy the anthers before any viable pollen is shed. More moderate conditions can extend the field life to hours.

Expression of Cry endotoxins confers resistance to insect feeding by certain lepidopterous larvae and, in theory, this would bestow an advantage on these transformed plants if they were heavily infested with herbivorous insects susceptible to *Bt* For these plants to become weedy escapes as a result of the genetic modification (i.e., insect resistance), they would need to inherit and express many other unrelated traits that provide selective advantage to a weedy growth habit (e.g., large numbers of easily dispersed seeds, propensity to grow on disturbed ground, vegetative propagation, seed dormancy, etc.). These traits do not exist within the maize complement of genetic characters, a species which has been selected for domestication and cultivation under conditions not normally found in natural settings. The presence of a large cob or ear that does not shatter as the bearer of seeds severely limits the dispersing abilities of maize and it has been theorized that in the absence of human intervention the species as we know it would die out in a few generations due to competition amongst seedlings germinating from the cob.

Transformation of corn to express *Bt* endotoxin does not alter the ability of maize to outcross with teosintes (*Zea mays* ssp. *mexicana*, *Z. mays* ssp. *parviglumis*, *Z. luxurians*, *Z. perennis*, *Z. diploperennis*) or *Tripsacum* species. Teosintes exist as special plantings (*e.g.*, in research plots, botanical gardens, and greenhouses) and some are used to a small extent as forage crops in the western United States. Many native teosintes in Mexico, El Salvador, Guatemala, Nicaragua and Honduras are interfertile with maize to varying degrees and have been known to produce viable seedlings. Despite having coexisted and co-developed in close proximity to maize in the Americas over thousands of years, however, maize and teosintes maintain distinct genetic constitutions even with this sporadic introgression. Given the cultural and biological relationships of various teosinte species and cultivated maize over the previous millennia, it appears that gene exchange has occurred (based largely upon morphological characters) between these two groups of plants and that no weedy types have successfully developed as a result. More recent cytogenetic, biochemical and molecular analysis has indicated that the degree of gene exchange is far less than previously thought and evidence for gene introgression into teosinte from maize may be considered as circumstantial at present.

The teosintes retain a reduced cob-like fruit/inflorescence that shatters more than cultivated maize, but still restricts the movement of seeds as compared to more widely dispersed weedy species. Hence, the dispersal of large numbers of seeds, as is typical of weeds, is not characteristic of teosintes or maize. In their native habitat, some teosintes have been observed to be spread by animals feeding on the plants. Teosintes and teosinte-maize hybrids do not survive even mild winters and would not propagate in the U.S. Corn Belt. Additionally, some types have strict day length requirements that preclude flowering within a normal season (i.e., they would be induced to flower in November or December) and, hence, seed production under our temperate climate.

Based on the ability of maize to hybridize with teosintes, the results of previous genetic exchange amongst these species over millennia, and their general growth habits, any introgression of genes into wild teosinte from *Zea mays* is not considered to be a significant agricultural or environmental risk. The growth habits of teosintes are such that the potential for serious weedy propagation and development is not biologically plausible in the United States.

Sixteen species of *Tripsacum* are known worldwide and generally recognized by taxonomists and agrostologists. Most of the 16 different *Tripsacum* species recognized are native to Mexico, Central and South America, but three occur within the U.S. The Manual of Grasses of the United States reports the presence of three species of *Tripsacum* in the continental United States: *T. dactyloides*, *T. floridanum* and *T. lanceolatum.* Of these, *T. dactyloides*, Eastern Gama Grass, is the only species of widespread occurrence and of any agricultural importance. It is commonly grown as a forage grass and has been the subject of some agronomic improvement (i.e., selection and classical breeding). *T. floridanum* is present in southern Florida and *T. lanceolatum* is present in the Mule Mountains of Arizona and possibly southern New Mexico.

For the species occurring in the United States, *T. floridanum* has a diploid chromosome number of 2n = 36 and is native to Southern Florida. *T. dactyloides* includes 2n = 36 forms which are established in the central and western U.S., and 2n = 72 forms which extend along the Eastern seaboard and along the Gulf Coast from Florida to Texas, but which have also been found in IL and KS; these latter forms may represent tetraploids (x = 9 or 18). *T. lanceolatum* (2n = 72) occurs in the Southwestern U.S. Eastern Gama Grass (*T. dactyloides*) differs from corn in many respects, including chromosome number (*T. dactyloides* commonly n = 18; *Zea mays* n = 10). Many species of *Tripsacum* can cross with *Zea*, or at least some accessions of each species can cross, but only with difficulty and the resulting hybrids are primarily male and female sterile.

T. dactyloides, is considered by some to be an ancestor of *Zea mays* or cultivated maize, while others dispute this, based largely on the disparity in chromosome number between the two species, as well as radically different phenotypic appearance. Albeit with some difficulty, hybrids between the two species have been made. In most cases these progeny have been sterile or viable only by culturing with *in vitro* "rescue" techniques. Relatively few accessions of *T. dactyloides* will cross with maize and the majority of progeny aren't fertile or viable even in those that do. In controlled crosses, if the female parent is maize, there is a greater likelihood of obtaining viable seed. When these hybrids have been backcrossed to maize in attempts to introgress *Tripsacum* genes for quality enhancement or disease resistance, the *Tripsacum* chromosomes are typically lost in successive generations.

Even though some *Tripsacum* species occur in areas where maize is cultivated, gene introgression from maize under natural conditions is highly unlikely, if not impossible. Hybrids of *Tripsacum* species with *Zea mays* are difficult to obtain outside of the controlled conditions of laboratory and greenhouse. Seed obtained from such crosses are often sterile or progeny have greatly reduced fertility. Approximately10 - 20% of maize-*Tripsacum* hybrids will set seed when backcrossed to maize, and none are able to withstand even the mildest winters. The only known case of a naturally occurring *Zea* - *Tripsacum* hybrid is a species native to Guatemala known as *Tripsacum andersonii.* It is 100 % male and nearly 99% female sterile and is thought to have arisen from an outcrossing to a teosinte, but the lineage is uncertain. *Zea mays* is not known to harbor properties that indicate it has weedy potential and other than occasional volunteer plants in the previous season=s corn field, maize is not considered as a weed in the U.S. The risk of *Tripsacum* / corn hybrids forming in the field is considered minimal. *Tripsacum* species are perennials and seem more closely related to the genus *Manisurus* than either to corn or teosinte.

Since both teosinte and *Tripsacum* are included in botanical gardens in the U.S., the possibility exists (although unlikely) that exchange of genes could occur between corn and its wild relatives. EPA is not aware, however, of any such case being reported in the United States. Gene exchange between cultivated corn and transformed corn would be similar to what naturally occurs at the present time within cultivated corn hybrids and landraces. Plant architecture and reproductive capacity of the intercrossed plants will be similar to normal corn, and the chance that a weedy type of corn will result from outcrossing with cultivated corn is extremely remote. Like corn, *Zea mays* ssp. *mexicana* (annual teosinte) and *Zea diploperennis* (diploid perennial teosinte) have 10 pairs of chromosomes, are wind pollinated, and tend to outcross, but are highly variable species which are often genetically compatible and interfertile with corn. *Zea perennis* (perennial teosinte) has 20 pairs of chromosomes and forms less stable hybrids with maize. Corn and compatible species of teosinte are capable of hybridization when in proximity to each other. In Mexico and Guatemala, teosintes exist as weeds around the margins of corn fields. The F1 hybrids have been found to vary in their fertility and vigor. Those that are fertile are capable of backcrossing to corn. However, except for special plantings as noted above, teosinte is not present in the U.S. or its territories. Its natural distribution is limited to Mexico, Honduras, Nicaragua and Guatemala. *Tripsacum*/maize hybrids have not been observed in the field, but have been accomplished in the laboratory using special techniques under highly controlled conditions.

C. ECOLOGICAL EFFECTS

A review of the studies submitted for the registration of the *B. thuringiensis* Cry1Ab 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 Cry1Ab delta-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 and an observable deleterious effect on the soil ecosystem is not expected to result from the growing of Cry1Ab delta-endotoxin-containing corn plants.

1. Avian Studies

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 Cry1Ab protein, and the low potential for chronic exposure of birds to significant concentrations of Cry1Ab.

2. 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 Cry1Ab protein produced in corn. Ciba Seeds determined through bioassay testing using the ECB, that a sample fish diet made using corn containing the *Bt* delta-endotoxin did not adversely affect ECB larvae and an analysis using ELISA indicated that Cry1Ab was not detectable in the feed samples. Therefore, fish eating a food mix made from corn containing the *Bt* delta-endotoxin would not be exposed to detectable active *Bt* delta-endotoxin protein.

3. 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* Cry1Ab endotoxin is a potential concern. However, in light of the above toxicology information, risk to mammalian wildlife is expected to be minimal to nonexistent.

4. Aquatic Invertebrate Studies

A 48-hour static renewal toxicity study of maize pollen containing *Bt* Cry1Ab delta-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 no adverse effects were observed at a maximum hazard dose of *Bt* Cry1Ab delta-endotoxin expressed in corn. In view of the above results, no freshwater aquatic invertebrate hazard is expected from the use of this product.

5. Estuarine and Marine Animal Studies

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

6. Nontarget Plant Studies

Since the active ingredient 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.

7. Honey Bee Studies

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

8. Non-Target Beneficial Organism Studies

a. Lady beetle predator

This study was scientifically sound and demonstrates that corn pollen containing the Cry1Ab 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.

b. Earthworm

This study was scientifically sound and demonstrated no significant treatment effects for Cry1Ab protein toxin to earthworms.

c. Collembola

This study was scientifically sound and demonstrates that the Cry1Ab 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.

d. Green lacewing

EPA recently performed a formal review of two Swiss National Science Foundation studies on the effects of *Event 176 Bt* corn and pure *Bt* corn toxin on lacewing. While the authors report detrimental effects on lacewing larvae from consumption of *Bt* corn toxin, their data show that lacewing mortality and developmental effects more likely are related to the study diet, not to any potential *Bt* endotoxin effects. Moreover, even if the reported results are taken at face value, the adverse effects are so slight as to suggest no significant impact on a substantial number of individual beneficial insects in a population in the field.

Hilbeck, *et al.*, report slightly elevated mortality and prolonged development time in lacewing larvae reared on *Bt* intoxicated prey (the European corn borer - ECB). The experimental design of the study, however, did not permit a distinction between a direct effect due to the *Bt* toxin on the predator versus an indirect effect of consuming a suboptimal diet consisting only of sick or dying prey that had succumbed to the *Bt* toxin. The dead or dying prey may have been septicemic (and therefore indirectly toxic), of limited nutritional value, or unpalatable to the lacewing. The lacewing was not given a choice in diet, which it has in a field setting. In nature the lacewing does not rely upon a single food source for development. In addition, the study has a high control mortality (34%, which is indicative of an unhealthy test system) and no prey consumption data. Also, there was no control with the purified *Bt* endotoxin. Generally, the findings are inconclusive and the laboratory report results are not directly transferable to the field use setting. The authors conclude that "...trials investigating predation efficiency and predator performance under field conditions are necessary before conclusions regarding the potential ecological relevance of the results presented in our paper can be drawn." [1] In addition, all available Agency in-house and published field data do not show significant detrimental effects due to *Bt* endotoxin on the lacewing.

Moreover, the authors subsequently reassessed the results of the study on reproductive effects on beneficial non-target organisms exposed to *Bt* corn in the laboratory.[2] According to Hilbeck, there are no significant reproductive effects from *Bt* corn toxin. The authors thus conclude that "[s]urviving, unaffected *C. carnea* developed at rates similar to those in the untreated control" and "[f]rom this, we conclude that total developmental time until adult eclosion is not an appropriate parameter for detecting Cry1Ab toxin effects."[3]

The second study used defined quantities of pure *Bt* toxin and there was significant mortality only in an *artificial* diet test group, and no significant mortality when the artificial diet was supplemented with *E. kuehniella* eggs (a natural diet).[4] Therefore, this study does not demonstrate any adverse effects to lacewing larvae under simulated field feeding habits where the lacewing larvae have a choice of natural diet in the field. Moreover, in this study, the concentration of pure Cry protein to which the larvae were exposed was massive (100 micro gm/ml of diet) and continuous, and therefore not reflective of Cry1Ab exposures that may occur under field conditions - either by exposure to plant tissues, pollen or by consumption of exposed prey species, such as ECB larvae. The dosage used in these studies is at least 30 times that found in most corn tissues in the field. Also, since in the field setting the lacewing larvae have a choice of other insects or eggs to feed on, field exposure will be intermittent, rather than continuous. Furthermore, in high-dose *Bt* corn fields intoxicated insects such as the ECB will not be available to the lacewings, since the ECB will be practically eliminated early by the *Bt* toxin in corn plants.[5] In addition, any surviving ECB larvae would normally be within the corn plant most of their larval life and not available for consumption by chrysopids.[6]

As noted in EPA's review of the first study, the lack of quantification of *Bt* consumption by the larvae makes it impossible to determine correlation between exposure to *Bt* and the observed responses. No data were presented to show the amount of prey consumed by each test group to make an independent assessment of unpalatability and sick prey effects that might be the result of food avoidance. The same is also true of the second study, in that it is not reported how much of a reduction in consumption of *Bt* toxin occurred in the replicates receiving a choice in diet. It is clear in this study also that there is a detrimental effect of the artificial study diet because data are presented that show an increase in mortality and development time in larvae reared exclusively on an artificial diet. Thus, the results of these studies do not support the conclusion that the *Bt* toxin was directly responsible for the observed differences in lacewing mortalities.

Environmental influences were also not considered in the speculation that *Bt* corn may pose a risk to beneficial insects. In a field setting it is highly improbable that lacewing larvae will mature exclusively on a diet of prey larvae that have been exposed to *Bt* endotoxin. Therefore it is highly unlikely that in the field the lacewings, or other beneficial insects, will ingest the amounts of *Bt* that the larvae were forced to consume in the laboratory study (i.e. there is a very low field exposure to the *Bt* toxin). The reported laboratory findings are not representative of the feeding environment by predatory insects in the open ecosystem, nor is the exposure to *Bt* endotoxin consistent with exposure that would be expected in the field.[1,5,7] Thus, on the basis of these considerations, EPA does not, at present, believe that additional data are necessary.

In general the reported laboratory findings do not show significant detrimental effects and do not provide data that show a risk to beneficial insects in a field use situation. The author, A. Hilbeck, agrees with this by stating that "...trials investigating predation efficiency and predator performance under field conditions are necessary before conclusions regarding the potential ecological relevance of the results presented in our paper can be drawn."[8] Moreover, there are published field studies on the effects of *Bt* crops on insect predators showing no significant differences in the density of beneficial insects.[9] These published field testing results and field test data submitted to EPA show minimal to undetectable changes in the beneficial insect abundance. Some actually report the densities of predatory and non-target insects as generally higher on transgenic than non-transgenic crops. To date the available field test data show that compared to crops treated with conventional chemical pesticides, the transgenic crops have no detrimental effect on a substantial number of individuals in beneficial insect populations.

9. Non-Target Insect Field Study

A study was submitted voluntarily by the registrant to determine whether *Bt* plant-pesticide maize, expressing truncated *Bt*-derived Cry1Ab delta-endotoxin, had a significant negative impact on natural non-target insect populations. It was shown that after spraying with a chemical insecticide, the number of insects caught in traps was significantly lower than the controls or Bt-expressing corn plants. No differences in insect numbers were found between the control plots and the Bt-expressing plants. However, the plots were very small and contained only 28 plants each. This small size could potentially lead to a great deal of variability and could reduce the significance of the results. The study is considered supplemental.

10. Endangered Species and Non-Target Lepidopteran Considerations

In the *Bacillus thuringiensis* (*Bt*) Reregistration Eligibility Decision document (RED), which considered the eligibility of *Bt* delta endotoxin in microbial sprays for reregistration, the Agency assessment concluded that these *Bt* microbial sprays "may affect" non-target lepidopteran insects. The RED "may affect" conclusion is based on published literature, especially on field data from the extensive use of *Bt* sprays in forests for gypsy moth control. The published field data is sufficient to assign hazard to all 750 US butterfly species without separate individual species testing. The field data show a temporary reduction in lepidopteran populations during prolonged *Bt* delta-endotoxin use, with population recovery after cessation of exposure to *Bt*.

Because the toxicity of *Bt* Cry proteins to butterflies is a well known and a widely published phenomenon, EPA risk assessments of *Bt* products have relied on lepidopteran (butterflies and moths) exposure to *Bt*. Since the exposure to butterflies and moths from the agricultural uses of *Bt* was not expected to be as high as in forest spraying (where no widespread/recurring or irreversible harm to lepidopteran insects was observed) , *Bt* corn likewise was not expected to cause widespread or irreversible harm to non-target lepidopteran insects.

In 1999, the following reports became available to the Agency regarding the effect of Novartis' Event 176 and Bt11 *Bt* corn pollen, respectively, on Monarch butterflies: (1) Hansen, L., and J. Obrycki.1999. Non-target Effects of *Bt* Corn Pollen on the Monarch Butterfly (Lepidoptera:Danaidae) Iowa State University, Ames , IA 50011. Presented at: North Central Branch meetings of the Entomological Society of America on March 29, 1999. Type: Poster Number: D81; and (2) John E. Losey, Linda S. Rayor, Maureen E. Carter, and Margaret E. Smith. 1999. Negative impact of transgenic pollen on monarch butterflies. Department of Entomology, Department of Plant Breeding, Cornell University, Ithaca, NY 14853. Draft Publication. Published as: John E. Losey, Linda S. Rayor, Maureen E. Carter. 1999. Transgenic pollen harms Monarch larvae. Nature. Vol. 399. 20 May 1999, p. 214.

The preliminary controlled study data are not useful for risk assessment of widespread or recurring *Bt* corn pollen effects on monarch butterflies without additional field study information.

Reports of toxicity of high doses of *Bt* to monarchs in the laboratory do not translate into exposure to toxic levels in the field. Further, the monarch butterfly is neither an endangered nor threatened species. It is an abundant and widespread insect which in North America ranges from central Mexico to southern Canada. There are many factors that cause severe mortality of monarchs, among these are predation, parasitism, destruction of the overwintering habitat, and most notably, climactic variations. However, since the publication of the Nature article, EPA has taken a number of steps to more fully assess and understand the possible risks to monarch butterflies and other butterflies, such as the endangered

Karner Blue butterfly, from *Bt* corn pollen. To help identify actual risks to non-target butterflies and moths, EPA has issued a data call-in to the registrants of *Bt* corn products under its FIFRA Section 3(c)2 (B) authority on December 15, 1999. The data required are listed below. Protocols are due in March 2000 and data are due in March 2001. If unreasonable risks are identified, EPA will take appropriate precautionary steps to reduce the risk to Monarch butterflies and other non-target butterflies and moths. EPA has also required Novartis and Mycogen to convey the following instructions via the Grower Guides/Product Use Guides or supplemental informational material provided to growers:

> "The potential for non-target species (e.g., monarch butterfly larvae) to be affected by *Bt* corn pollen remains under study. As an interim measure, the EPA is encouraging growers to place the non-*Bt* corn refuge between *Bt* corn and habitats such as prairies, forests, conservation areas, and roadsides."

The following are the data required under the Bt corn Data Call-In:

(a) Determine and report (in square miles) the total land mass in North America that contains milkweed and monarchs vs. the total amount of land at the edge of corn fields where milkweed could be exposed to *Bt* corn pollen.

(b) Determine and report what species of milkweed monarchs feed on.

(c) Determine and report what percentage of milkweed in the cornbelt is found in row crop areas vs. roadsides, pastures and other non-row crop areas.

(d) Provide surveys of corn fields in representative corn growing states to determine how much milkweed is in the fields.

(1) Determine and report whether milkweeds are closer, farther or at random distance to corn.
(2) Provide data on the relative abundance of milkweed in the corn field pollen shadow verses areas further than 60 meters away from corn fields.
(3) Determine and report whether herbicides are effective in corn fields in eliminating milkweeds. If so, determine which herbicides are most effective.

(e) Determine and report what is the relationship between monarch colonization of milkweeds and distance to corn.

(1) Determine and report the distribution of monarch eggs and larvae on milkweeds relative to corn fields.
(2) Quantify and report the pollen on milkweed leaves within the pollen shadow and up to 60 meters from the edge of *Bt* corn fields.
(3) Provide the distances from the edge of corn field at which LD50 concentrations of *Bt* pollen are found for each *Bt* corn hybrid.

(f) Determine and report the LD50s for the Cry protein in your *Bt* corn active ingredient(s) for a) monarch larvae and for b) larvae of a relative of the endangered Karner Blue butterfly. The Karner Blue butterfly relative tested must be from the genus Lycaeides, such as the Northern Blue butterfly (*Lycaeides idas*). If it is not feasible to test a butterfly from the genus Lycaeides, then you must provide justification regarding why such testing is not feasible and test a butterfly from a genus within the family Lycaenidae. The Karner Blue butterfly must not be tested.

(g) Determine the monarch larvae LD50s for pollen, for representative inbreds and hybrids from your transformation event(s), containing your *Bt* corn plant-pesticide and report the results on both a weight and a number of pollen grains basis.

(h) Determine and report each instar larval survival and developmental effects in the presence of *Bt* pollen, for representative inbreds and hybrids from your transformation event(s), containing your *Bt* corn plant-pesticide.

(i) The Cornell data (Losey, J., L. Raynor, and M. Carter. 1999. Transgenic pollen harms monarch larvae. Nature 399:214.) show that less larval feeding took place on pollinated milkweed leaves than on non-pollinated leaves. Therefore:

(1) Determine and report what is the probability of corn pollen consumption by monarch larvae on Milkweed leaves;
(2) Determine and report whether foraging larvae actively avoid *Bt*-pollen, for representative inbreds and hybrids from your transformation event(s); in the field;
(3) Determine and report whether monarch larvae avoid feeding on non-*Bt* corn pollen under field conditions;
(4) Determine and report whether monarchs are avoiding corn fields for preferred areas to feed.

(j) Determine and report whether there are practical ways of decreasing the potential of monarchs encountering or feeding upon *Bt* pollen.

(k) Determine and report whether monarchs have a site preference for egg laying;

(1) Determine and report whether monarch adults oviposit on or avoid milkweeds near corn fields.

(l) Determine and report what is the effect of *Bt* corn pollen presence, for representative inbreds and hybrids from your transformation event(s), on monarch oviposition behavior;

(1) Determine and report whether monarchs deposit eggs on non-*Bt* corn pollinated milkweed under field conditions,
(2) Determine and report whether monarch adults deposit eggs on *Bt* corn pollinated milkweed under field conditions,
(3) Determine and report where monarch adults oviposit on milkweeds (under leaves, in inflorescents).

(m) Confirm and report where the various instars of monarch larvae feed on the milkweed plant:

(1) upper vs. lower leaves,
(2) also determine and report on feeding behavior regarding upper and undersides of leaves (changes potential exposure considerably),
(3) shoot apex vs tops of leaves, and
(4) determine and report whether pollen will disseminate to and adhere to the undersides of leaves.

(n) Determine and report how long the lethal concentration of *Bt* corn pollen, for representative inbreds and hybrids from your transformation event(s), stays on milkweed.

(1) Determine and report how long *Bt* in corn pollen retains its toxicity,
(2) Determine and report whether sunlight degrades *Bt* toxin in corn pollen on milkweed, and
(3) Determine and report whether wind, rain or other environmental factors remove *Bt* corn pollen from milkweed.

(o) Determine and report how soon after planting do representative inbreds and hybrids from your transformation event(s) pollinate.

(p) Determine and report whether the duration of pollination for each corn ear and the total field match the expected 3 and 13 days, respectively, for representative inbreds and hybrids from your transformation event(s).

(q) Determine and report whether the monarch larvae are feeding on milkweeds during pollen shed.

(1) If so, determine and report how long is the regional overlap of time when the monarch larvae are exposed to corn pollen.
(2) Determine and report what fraction of monarch larvae could be exposed to corn pollen, considering that in any specific region the corn is shedding pollen for only a week to ten days each year.
(3) Determine and report the probability of monarch larvae encountering pollen from *Bt* corn

(r) Determine and report whether monarchs carry pollen on their exoskeleton and distribute it on milkweeds during egg deposition. If so, determine the quantity.

(s) Determine and report what is the risk of monarch exposure to *Bt* corn pollen in the context of other significant risk factors impacting monarch survival and population size (e.g. conventional and microbial insecticides, herbicides, destruction of overwintering sites, predation, cars, etc).

(t) Determine and report whether monarch populations travel linearly.

(u) Confirm and report whether 50% of monarchs pass through the corn belt.

(v) Develop and report a mathematical model to test the sensitivity of various environmental and biological risk factors, as well as to examine the risk to monarchs and other susceptible non-target insects at varying distances from *Bt* corn fields.

(w) Define and report baseline monarch population levels and submit annual population level reports on a regional basis.

D. INSECT RESISTANCE MANAGEMENT

Bt insect resistance management (IRM) is of great importance because of the threat insect resistance poses to the future use of *Bt* pesticides. Public interest groups and organic farmers have expressed concern that the widespread planting of these genetically transformed plants will hasten the development of resistance to pesticidal *Bt* endotoxins.

To address this real concern, EPA has imposed IRM requirements on registered *Bt* plant-pesticides. Sound IRM will prolong the life of *Bt* pesticides and universal adherence to the plans is to the advantage of growers, producers, and researchers alike. EPA's strategy to address insect resistance is two-fold: (1) mitigate any significant potential for pest resistance development in the field by instituting IRM plans, and (2) better understand the mechanisms behind pest resistance.

1. Bt Field Corn

Beginning with the first *Bt* plant-pesticide registration, the Agency has taken steps to manage insect resistance to *Bt* with IRM plans being an important part of the regulatory decision. These mitigation measures include IRM plans to prevent or manage resistance, field research and resistance monitoring, establishing refuge (a portion of the total acreage using non-*Bt* seed), and appropriate changes in the plans as more information becomes available. It is believed that planting refuge will delay the development of insect resistance by maintaining insect susceptibility.

Bt corn crops express one of three registered *Bt* endotoxins, Cry1Ab, Cry1Ac, or Cry9C in either field corn (grown primarily for non-human animal consumption), sweet corn or popcorn (the latter two grown primarily for human consumption). EPA has used the best available scientific information in its IRM assessment and has updated its IRM position as information has become available. EPA will continue to use science-based decision-making as it reevaluates IRM requirements for *Bt* corn, the registrations of which expire in April, 2001.

Bt corn presents an additional concern related to pests that are polyphagous, i.e., pests that feed on more than one crop. The corn earworm (CEW)/cotton bollworm (CBW) (*Helicoverpa zea*) is an example of a polyphagous pest. CEW/CBW is a pest of both corn and cotton and early generations may live in corn with subsequent generations in cotton during one growing season. It is possible that as many as six generations of CEW/CBW can be exposed to the same or related *Bt* toxins expressed in *Bt* corn and *Bt* cotton, significantly increasing the likelihood of the development of resistance. Because CEW/CBW also feeds on other crops (e.g., soybean and tomato), there is also an increased potential for resistant CEW/CBW to move to other host crops that may be treated with *Bt* foliar sprays, thus rendering the *Bt* ineffective.

In 1995, at the time of the initial registrations of *Bt* corn, there was no scientific consensus on the details of the IRM plans necessary for prevention of the development of resistance in the two primary target pests, European corn borer (*Ostrinia nubilalis* (Hübner)), ECB) and corn earworm (CEW). At that time, the putative values for adequate refuge size ranged from 20% to 50% of non-*Bt* corn or other host plants per farm. While the minimum adequate refuge size or structure could not be determined until further research was conducted, it was thought that market penetration of these crops would be sufficiently slow that considerable non-*Bt* corn would remain to act as natural refuges while the additional research was conducted. Thus, the initial *Bt* corn registrants instituted voluntary IRM plans. The registrants agreed to various voluntary refuge requirements in the Corn Belt. For example, Mycogen indicated a commitment to develop a long-term insect resistance management strategy, provide general insect resistance management "guidance," and recommended that not all corn acres be planted in *Bt* corn. Novartis indicated a commitment to develop a long-term insect resistance management strategy, provided general insect resistance management "guidance," and informed growers that part of a long-term insect resistance management strategy may be "the maintenance of a refuge where susceptible populations of ECB can escape exposure" to the expressed *Bt* endotoxin. However, EPA restricted the sale or distribution of *Bt* Cry1Ab and Cry1Ac corn products in certain southern counties and states where most cotton is grown. These sales restrictions were necessary to mitigate the development of resistance to *Bt* toxins in CEW/CBW populations feeding on both corn and cotton. EPA also requested data to develop appropriate refuge options for areas in which corn and cotton are grown.

Since 1995, all *Bt* corn registrations have included a resistance monitoring plan for ECB and CEW (except for Cry9C) that contained the following elements: (1) development of baseline susceptibility responses and a discriminating concentration to detect changes in sensitivity, (2) routine surveillance, and (3) remedial action if there is suspected resistance. The purpose of resistance monitoring is to learn whether a field control failure resulted from resistance or other factors that might inhibit expression of the *Bt* Cry delta endotoxin. The extent and distribution of resistant populations can be mapped and alternative control strategies implemented in areas in which resistance has become prevalent. If monitoring techniques are sensitive enough to discriminate between resistant and susceptible individuals, it should be possible to detect field resistance before significant loss of efficacy and eliminate any resistant individuals using other control tactics. In addition, EPA mandated that all registrants must require customers to notify them of incidents of unexpected levels of ECB and CEW damage. Registrants are required to investigate these reports and identify the cause of the damage by local field sampling of the plant tissue and suspect insect populations followed by appropriate *in vitro* and *in planta* assays. Any confirmed incidents of resistance are required to be reported to EPA. Based on these investigations, appropriate remedial action is required to mitigate ECB and/or CEW resistance. These remedial actions include: informing customers and extension agents in the affected areas of ECB and/or CEW resistance, increasing monitoring in the affected areas, implementing alternative means to reduce or control ECB or CEW populations in the affected areas, implementing a structured refuge in the affected areas, and cessation of sales in the affected and bordering counties. All registrants have instructed growers to have regular surveillance programs and report any unexpected levels of ECB and CEW damage.

The Novartis 1997 technical bulletin for Cry1Ab hybrids encouraged growers to: (1) plant *Bt* hybrids in large blocks, (2) scout for non-target pest and use IPM strategies, (3) maintain a refuge of non-*Bt* corn, and (4) monitor for unexpected levels of insect damage in *Bt* corn. In 1998, Novartis recommended that growers follow the IRM guidance for a 20-30% unsprayed structure refuge and a 40% refuge is sprayed with insecticides as outlined in the USDA North Central Regional Publication 602.

In February 1998, EPA requested that the FIFRA SAP subpanel on *Bt* plant-pesticide resistance management review existing IRM strategies for *Bt* crops. Following the recommendations of this SAP subpanel, EPA began to mandate specific structured refuge options for new *Bt* corn registrations (those products registered prior to that time were still expected to implement voluntary refuge options). The specific structured refuge requirements were based on the technical recommendations of the February 1998 FIFRA SAP subpanel and USDA NC-205 research committee on ecology and management of European corn borer and other stalk-boring Lepidoptera (NC-205). The NC-205 regional research committee published IRM recommendations in 1997 and 1998. In 1998, NC-205 recommended at least a 20-30% untreated refuge or 40% treated refuge planted within close proximity (<320 acre section of *Bt* corn).

In 1998, EPA required that Novartis mandate (through grower contracts) a 20-30% unsprayed refuge, or, if treated with non-*Bt* insecticides, a 40% refuge planted within 0.5 miles of *Bt* corn fields for its Cry1Ab popcorn.

For the 1999 growing season, the Novartis Event 176 Cry1Ab field corn registration, the Novartis 1999 grower guide instructed growers to plant a 20% non-*Bt* corn refuge that may be treated with non-*Bt* insecticides. For the Mycogen Event 176 Cry1Ab field corn registration, the Mycogen 1999 grower guide instructed growers to plant a 20% untreated non-*Bt* corn refuge or if treated with non-*Bt* insecticides, a 40% non-*Bt* corn refuge.

As part of the original terms and conditions of registration mandated in 1995, EPA required that draft IRM plans be submitted by August 1998 for review, be finalized in 1999, and be implemented in 2001 (registrations expire April 1, 2001). Draft refuge strategies for all Cry1Ab and Cry1Ac field corn and popcorn products were submitted to EPA in August 1998. In April 1999, registrants submitted final refuge strategies for Cry1Ab and Cry1Ac field corn products developed in association with the National Corn Growers Association (NCGA) plan. The industry/NCGA plan focuses on the implementation of a 20% refuge that may be treated if the level of pest pressure meets or exceeds economic thresholds. The plan encourages planting of the non-*Bt* corn refuge within one-quarter mile of the *Bt* corn acreage where feasible, and requires planting the refuge within one-half mile of the *Bt* corn acreage. If treatment of the refuge is expected, the plan requires planting of the refuge within one-quarter mile of the *Bt* corn plantings. The plan also stated that a 20% untreated refuge or 40% refuge, if treated, should be planted in Northern cotton areas and a 50% refuge that may be treated should be planted in Southern cotton areas. In May 1999, NC-205 reviewed the April 1999 industry/NCGA insect resistance management plan for all Cry1A field corn products and concluded that a 20% sprayed refuge may be adequate in most corn growing areas where economic thresholds for ECB are not regularly exceeded. NC-205 stated that a 20% infrequently sprayed refuge is acceptable. This would apply to most of the Corn Belt east of the High Plains region. NC-205 indicated, however, that further research regarding the efficacy of a 20% sprayed refuge was needed, especially in higher risk areas such as the High Plains region, in which insecticide use has been historically high. Field corn in the United States is rarely sprayed for ECB or CEW. Southwestern corn borer (*Diatraea grandiosella*, SWCB) is typically treated with insecticides. On average, only approximately 8% of total U.S. field corn acreage is treated for these pests. In those areas considered high risk, insect damage at or above economic thresholds is common and, thus growers use insecticides more often than elsewhere in the Corn Belt. NC-205 also noted that all *Bt* corn should be placed within one half mile of the non-*Bt* corn refuge, but that refuge plantings within one quarter mile would be even better.

For the year 2000, all *Bt* field corn products will have mandatory structured refuge requirements. EPA mandated that all Cry1Ab field corn products will either have a minimum 20% (treatable) non-*Bt* corn refuge in the Corn Belt or a minimum 50% (treatable) non-*Bt* corn refuge if *Bt* corn is grown in southern cotton-growing areas. Larger refuges (≥50% non-*Bt* corn) for *Bt* corn grown in southern cotton-growing areas are necessary to mitigate resistance development to *Bt* toxins in CBW/CEW populations feeding on both corn and cotton (both Cry1Ab and Cry1Ac registrations). The refuge must be planted within ½ mile of the *Bt* corn fields. In regions of the Corn Belt where conventional insecticides have historically been used to control ECB and SWCB, growers wanting the option to treat these pests must plant the refuge within 1/4 mile of their *Bt* corn fields. In addition, both registrants of non-high expression Event 176 corn products agreed to restrict sales of these products in areas which are routinely treated with insecticide sprays, notably the areas which are jointly infested with SWCB and ECB (mainly parts of Oklahoma, Kansas, Texas, and Colorado). Previous IRM requirements for Cry1Ab popcorn will remain for the 2000 growing season, i.e., substantial non-*Bt* corn refuge (20-30% unsprayed/40%, if sprayed) must be planted within 0.5 miles of the *Bt* popcorn fields.

In addition, for the 2000 growing season, specific regional monitoring plans must be expanded to include ECB, SWCB, and CEW and be submitted to the Agency by March 31, 2000. [*These plans have been submitted and are pending Agency review.*] Registrants must also conduct annual grower surveys to assess compliance with specific IRM requirements. These IRM requirements provide consistency amongst all Cry1A-expressing field corn products.

2. Bt Popcorn

As of 1996, the leading producer of popcorn in the Unites States is Indiana. From 1977-1981, 55% of all popcorn was produced in Nebraska and Indiana and 25% in Iowa, Ohio, and Illinois. During that five year period there was an average of 189,000 acres of popcorn produced per year. The 1992 Census of Agriculture, U.S. Department of Commerce, Bureau of the Census, states that there were 321,485 acres of popcorn harvested that year.

Most popcorn hybrids cannot be pollinated by ordinary types of field or sweet corn. Popcorn's life cycle most closely follows field corn but germination and seedling growth is slow and therefore popcorn pollinates at a different time than field corn. Seeding rates for popcorn are higher than for field corn because of its smaller plant size and lower yield per plant which also results in slightly different growth habits.

Most, if not all, of the insects that attack field corn can also attack popcorn. Soil insects include: northern and western corn rootworms, wireworms, grubs, and two species of cutworms. Foliar and stem insects include: aphids, corn earworm, fall armyworm, leaf beetles, mites, European corn borer, southwestern corn borer, and grasshoppers. The European corn borer (ECB) is the primary insect pest in popcorn.

Because popcorn is not grown in cotton-producing regions (where insect resistance is a greater concern) and there are only approximately 300,000 acres of popcorn planted annually in the United States (compared to an average of approximately 70 million acres of field corn), the addition of popcorn to the existing registration of Cry1Ab delta endotoxin and the genetic material necessary for its production in Event 176 field corn] will only pose a minimal additional ECB and corn earworm resistance risk.

Previous IRM requirements for Cry1Ab popcorn will remain for the 2000 growing season, i.e., substantial non-*Bt* corn refuge (20-30% unsprayed/40%, if sprayed) must be planted within 0.5 miles of the *Bt* popcorn fields.

IV. DETAILS REGARDING THE TERMS AND CONDITIONS OF THE USES

A. FIELD CORN COMMERCIAL USE

The terms and conditions of this use require the following:

1. Submission of production information for these products for the fiscal year in which the use of commercial field corn is conditionally registered, in accordance with FIFRA § 29. The fiscal year begins October 1 and ends September 30. Production information will be submitted to the Agency no later than November 15, following the end of the preceding fiscal year.

2. The field corn use will automatically expire on midnight April 1 , 2001. EPA will reevaluate the effectiveness of Novartis Seeds (Field Crops) and Mycogen Seeds' resistance management plans before April 1, 2001 and decide whether to convert the registration to a

non-expiring registration.

3. For *Bt* field corn grown outside cotton-growing areas (e.g., the Corn Belt), grower agreements (Stewardship Agreements) will specify that growers must adhere to the refuge requirements as described in the Grower Guide/Product Use Guide and/or in supplements to the Grower Guide/Product Use Guide. Specifically, growers must plant a minimum structured refuge of at least 20% non-*Bt* corn. Insecticide treatments for control of European corn borer, corn earworm and/or Southwestern corn borer may be applied only if economic thresholds are reached for one or more of these target pests. Economic thresholds will be determined using methods recommended by local or regional professionals (e.g., Extension Service agents, crop consultants). Instructions to growers will specify that microbial *Bt* insecticides must not be applied to non-*Bt* corn refuges. In addition, *Bt* field corn under this registration must not be offered for sale after the close of business January 5, 2000 in the following counties for the four listed states:

Texas: Armstrong, Bailey, Carson, Castro, Collingsworth, Dallam, Deaf Smith, Donley, Floyd, Gray, Hale, Hansford, Hartley, Hutchinson, Lamb, Lipscomb, Moore, Ochiltree, Parmer, Potter, Randall, Roberts, Sherman, Swisher, Wheeler

Colorado: Baca, Bent, Cheyenne, Kiowa, Kit Carson, Otero, Prowers

Oklahoma: Beaver, Cimarron, Texas

Kansas: Barton, Edwards, Ellsworth, Finney, Ford, Grant, Gray, Hamilton, Harvey, Haskell, Hodgeman, Kearny, Kingman, Kiowa, Lane, McPherson, Meade, Morton, Ness, Pawnee, Pratt, Reno, Rice, Rush, Seward, Stafford, Stanton, Stevens

4. For the 2000 growing season, grower agreements (Stewardship Agreements) for *Bt* field corn grown in cotton-growing areas will specify that growers must adhere to the refuge requirements as described in the Grower Guide/Product Use Guide and/or in supplements to the Grower/Product Use Guide. Specifically, growers in these areas must plant a minimum structured refuge of 50% non-*Bt* corn. Cotton growing areas include the following States: Alabama, Arkansas, Georgia, Florida, Louisiana, North Carolina, Mississippi, South Carolina, Oklahoma (only the counties of Bryan, Caddo, Canadian, Garvin, and Grady), Tennessee (only the counties of Carroll, Chester, Crockett, Fayette, Franklin, Gibson, Hardeman, Hardin, Haywood, Hendersen, Lake, Lauderdale, Lawrence, Lincoln, McNairy, Madison, Obion, Rutherford, Shelby, and Tipton), Texas (except the counties of Carson, Dallam, Hansford, Hartley, Hutchinson, Lipscomb, Moore, Ochiltree, Roberts, and Sherman), Virginia (only the counties of Greensville, Isle of Wight, Northampton, Southampton, Sussex, Suffolk) and Missouri (only the counties of Butler, Dunkin, Mississippi, New Madrid, Pemiscot, Scott, Stoddard).

5. Requirements for refuge deployment will be described in the Grower Guides/Product Use Guides as described in Section D of the Industry IRM Plan submitted on April 19, 1999. Growers must continue to be required to plant only non-*Bt* corn in the refuge and to plant the refuge within ½ mile of their *Bt* corn acreage. In regions of the corn belt where conventional insecticides have historically been used to control ECB and SWCB, growers wanting the option to treat these pests must plant the refuge within ¼ mile of their *Bt* corn. Refuge planting options include: separate fields, blocks within fields (e.g., along the edges or headlands), and strips across the field. When planting the refuge in strips across the field, growers must be instructed to plant multiple non-*Bt* rows whenever possible.

6. Novartis Seeds (Field Crops) and Mycogen Seeds will monitor for the development of resistance using baseline susceptibility data and/or a discriminating concentration assay when such an assay is available. Novartis Seeds (Field Crops) and Mycogen Seeds will continue efforts to develop a discriminating concentration assay. Novartis Seeds (Field Crops) and Mycogen Seeds will ensure that monitoring studies are conducted annually to determine the susceptibility of ECB populations to the Cry1Ab 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, Novartis Seeds (Field Crops) and Mycogen Seeds will instruct their customers (growers and seed distributors) to contact Novartis Seeds (Field Crops) and Mycogen Seeds if incidents of unexpected levels of ECB damage occur. Novartis Seeds (Field Crops) and Mycogen Seeds 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 Novartis Seeds (Field Crops) and Mycogen Seeds' confirmation by immunoassay that the plants contain Cry1Ab 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, Novartis Seeds (Field Crops) and Mycogen Seeds 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 Cry1Ab 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 Cry1Ab 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 Cry1Ab-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 Novartis Seeds (Field Crops) and Mycogen Seeds find it appropriate to alter the criteria specified in the working definition, Novartis Seeds (Field Crops) and Mycogen Seeds must obtain Agency approval in establishing a more suitable definition.

7. The current insect monitoring programs must be expanded to include Southwestern corn borer (SWCB) and corn earworm (CEW), in addition to European corn borer (ECB). The expanded program must focus monitoring in areas that typically have a high density of *Bt* corn or have historically been prone to high levels of corn borer pressure and where the refuge areas may more likely be treated with insecticides. Plans for your modified monitoring plan must be provided to the Agency by March 31, 2000 for review. [These plans have been submitted and are pending Agency review.]

8. The current definition of confirmed insect resistance must be used as described in Section E of the Industry IRM Plan. Agency approval will be sought prior to implementation of any modified definition of confirmed insect resistance.

9. When resistance has been demonstrated to have occurred, the registrant must stop sale and distribution of *Bt* corn in the counties where the resistance has been shown until an effective local mitigation plan approved by EPA has been implemented. EPA understands that legal constraints will not allow the amendment of grower guides or agreements currently in effect to require remedial actions to be taken by the grower. Therefore, Novartis and Mycogen assume responsibility for the implementation of resistance mitigation actions undertaken in response to the occurrence of resistance during the 2000 growing season. EPA interprets "suspected resistance" to mean, in the case of reported product failure, that the corn in question has been confirmed to be *Bt* corn, that the seed used had the proper

percentage of corn expressing *Bt* protein, that the relevant plant tissues are expressing the expected level of *Bt* protein, that it has been ruled out that species not susceptible to the protein could be responsible for the damage, that no climatic or cultural reasons could be responsible for the damage, and that other reasonable causes for the observed product failure have been ruled out. The Agency does not interpret "suspected resistance" to mean grower reports of possible control failures, nor does the Agency intend that extensive field studies and testing to fully scientifically confirm insect resistance be completed before responsive measures are undertaken.

10. Novartis Seeds (Field Crops) and Mycogen Seeds 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. Novartis Seeds (Field Crops) and Mycogen Seeds 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.

11. Novartis Seeds (Field Crops) and Mycogen Seeds will provide grower education. Novartis Seeds (Field Crops) and Mycogen Seeds will include an active partnership with such parties as: university extension entomologists and agronomists, consultants, and corn grower groups. Novartis Seeds (Field Crops) and Mycogen Seeds will implement a grower education program directed at increasing grower awareness of resistance management, in order to promote responsible product use. IRM educational materials for the 2000 growing season must be provided to the Agency as they become available for distribution. Survey results and other available information must be used to identify geographic areas of non-compliance with insect resistance management plans. As described in the Industry IRM Plan, an intensified grower education program will be conducted in these geographic areas prior to the following growing season. If individual non-compliant growers are identified, they must be restricted from future purchases of *Bt* corn seed. You must convey the following instructions via the Grower Guides/Product Use Guides or supplemental informational material provided to growers:

> "The potential for non-target species (e.g., monarch butterfly larvae) to be affected by *Bt* corn pollen remains under study. As an interim measure, the EPA is encouraging growers to place the non-*Bt* corn refuge between *Bt* corn and habitats such as prairies, forests, conservation areas, and roadsides."

12. Several aspects of the IRM Plan will operate in synergy to promote grower compliance, however, the cornerstones of the compliance program must be the:

> a. Grower Guides These Guides must be distributed to each seed customer and updated on an annual basis, as needed. The Guides provide complete information for growers regarding routine IRM practices that must be employed, and will be a primary educational and reference tool. Agreed-upon requirements and additional information that cannot be included in the Grower Guides for 2000 (e.g., because the requirements were enacted after printing and distribution of the Grower Guides) must be conveyed via supplemental communications to *Bt* field corn seed customers.

> b. Stewardship Agreement (grower agreement).Each grower who purchases *Bt* field corn seed must be required to sign a Stewardship Agreement, which will obligate the grower to follow the required IRM practices as specified in the Grower Guide/Product Use Guide and/or in supplements thereof.

> c. A Strong and Multi-Pronged Grower Education Program.A variety of methods must be employed to promote grower education and to continue to reinforce the need for adherence to all aspects of the IRM program.

> d. Additional mechanisms must also be used to promote grower compliance,

including:

Training of sales personnel, seed dealers and technical support staff.

Coordination and reinforcement of IRM requirements through other organizations (e.g., NC-205, the Cooperative Extension Service, USDA, National Corn Growers Assn. (NCGA), American Crop Protection Assn., Biotechnology Industry Organization, crop consultants and other crop professionals).

13. Novartis Seeds (Field Crops) and Mycogen Seeds will confer with EPA as they develop various aspects of their individual IRM research 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 a. through g. of this section. Novartis Seeds (Field Crops) and Mycogen Seeds will conduct the research specified in paragraph a. through e. and any other research that is included in the program that EPA accepts. Novartis Seeds (Field Crops) and Mycogen Seeds will 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 both "block" refuge, "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 Cry1Ab delta-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.

f. The registrant must assess the feasibility of using the F2 screen, sentinel plots, and in-field screening kits to increase the sensitivity of resistance monitoring in 2000. By January 31, 2001, you must provide the Agency with the results from these investigations.

g. The registrant must implement a survey approach similar to the Iowa State University *Bt* Corn Survey (e.g., Pilcher and Rice, 1999*)* A statistically valid sample, as determined by independent market research, of *Bt* corn growers in key states will be surveyed by a third-party. *Bt* corn growers will be included based upon a proportionately stratified random sample designed to balance the survey evenly across seed companies and geographies. In addition to demographic information, the survey will include questions related to insect resistance management such as:

i) What is your primary source of information on *Bt* corn?
ii) What percentage of your acres were planted to *Bt* corn this year?
iii) Are you following a recommended insect resistance management strategy?
iv) If you plant most of your acreage to *Bt* corn, are you likely to

scout your non-*Bt* corn for economically damaging populations of corn borers?
v) Did you treat your *Bt* corn acres with an insecticide?
vi) What planting pattern did you use for your refuge?

° Planted *Bt* corn as one block in one field.
° Planted *Bt* corn in one block in every field.
° Split seed boxes in the planter and alternated every row or several rows with *Bt* and non-*Bt* corn in every field.
° Planted *Bt* corn in large strips alternated with large strips of a non- *Bt* corn hybrid.
° Planted *Bt* corn in an entire field and planted the border around the field with non-*Bt* corn.
° Planted pivot corners to non-*Bt* corn with the irrigated area of the field planted to *Bt* corn.

B. POPCORN COMMERCIAL USE

The terms and conditions for this use requires the following.

1. Submission of production information (80,000 seed units of seed produced) for this product (both popcorn and field corn) for each fiscal year in which this product is conditionally registered, in accordance with FIFRA ' 29. The fiscal year begins October 1 and ends September 30. Production information will be submitted to the Agency no later than November 15, following the end of the preceding fiscal year.

2. This registration will automatically expire on midnight April 1, 2001. EPA will reevaluate the effectiveness of Novartis Seeds (Field Crops)'s resistance management plans before April 1, 2001 and decide whether to convert the registration to a non-expiring registration.

3. Novartis Seeds (Field Crops) will do the following regarding structured refuge:

a. For the popcorn use, Novartis Seeds (Field Crops) and/or its licensees will require popcorn growers to sign a contractual agreement that binds the grower to implement a structured refuge for insect resistance management when planting Novartis Seeds' Event 176 *Bt* popcorn. The contract will state that popcorn growers who do not comply with this requirement will not be sold Event 176 *Bt* popcorn the following year;

b. For the popcorn use, the contractual agreement will state that popcorn growers must follow the recommendations for structured refuges presented in the NC 205 report prepared by the Research and Extension Entomologists of the North Central Regional Research Project (e.g., 20-30% of the corn acreage will be non-*Bt* corn, or in continuous corn areas where European corn borers are typically sprayed with insecticides, 40% of the corn acreage will be non-*Bt* corn; the refuge must be within 2 mile of the *Bt* popcorn fields). Specifically, the recommendations set forth in the NC-205 report will be followed (Ostlie, K.R., W.D. Hutchinson, and R.L. Hellmich, 1997. *Bt Corn and European Corn Borer.* NCR Publication 602);

c. For the popcorn use, the land that comprises the required refuge may consist of any non-*Bt* field corn or non-*Bt* popcorn acreage.

4. Novartis Seeds (Field Crops) will monitor for the development of resistance using baseline susceptibility data and/or a discriminating concentration assay when such an assay is available. Novartis Seeds (Field Crops) will continue efforts to develop a discriminating concentration assay. Novartis Seeds (Field Crops) will ensure that monitoring

studies are conducted annually to determine the susceptibility of ECB populations to the Cry1Ab 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, Novartis Seeds (Field Crops) will instruct their customers (growers and seed distributors) to contact Novartis Seeds (Field Crops) (e.g., via a toll-free customer service number) if incidents of unexpected levels of ECB damage occur. Novartis Seeds (Field Crops) 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 Novartis Seeds (Field Crops)'s confirmation by immunoassay that the plants contain Cry1Ab 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, Novartis Seeds (Field Crops) 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 Cry1Ab 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 Cry1Ab 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 Cry1Ab-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 Novartis Seeds (Field Crops) find it appropriate to alter the criteria specified in the working definition, Novartis Seeds (Field Crops) must obtain Agency approval in establishing a more suitable definition.

5. Novartis Seeds (Field Crops) 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 Novartis Seeds (Field Crops) 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, Novartis seeds (field crops) 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, Novartis seeds (field crops) will voluntarily cease sale of all of Novartis seeds (field crops)' Cry1Ab 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, Novartis Seeds (Field Crops) 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, Novartis Seeds (Field Crops) can resume sales in the affected county (ies).

6. Novartis Seeds (Field Crops) will maintain a database to track its sales (units and location) of Event 176-derived popcorn on a county-by-county basis, to the extent that such data are available. Novartis Seeds (Field Crops) 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. Novartis Seeds (Field Crops) must provide grower education. Novartis Seeds (Field Crops) will include an active partnership with such parties as: university extension entomologists and agronomists, consultants, and corn grower groups. Novartis Seeds (Field Crops) 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. Novartis Seeds (Field Crops) will inform the Agency as they develop, implement, and refine their communication strategies. In addition to grower communication vehicles, Novartis Seeds (Field Crops) 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. Novartis Seeds (Field Crops) must develop a resistance management program that is acceptable to EPA and that includes the research specified in paragraph a. through e. of this section. Novartis will confer with EPA as it develops 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 a. through e. of this section. Novartis Seeds (Field Crops) must conduct the research specified in paragraph a. through e., and any other research that is included in the program that EPA accepts. Novartis Seeds (Field Crops) will use the research as a basis to develop a long-term resistance management strategy.

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DISCLAIMER: *The information in this Pesticide Fact Sheet is a summary only and is not to be used to satisfy data requirements for pesticide registration. Contact the Regulatory Action Leader listed above for further information.*

Endnotes

1. Hilbeck, A., Moar, W.J., Pusztai-Carey, M., Fillippi, A., and F. Bigler, *Toxicity of Bacillus thuringiensis CryIAb Toxin to the Predator Chrysoperla carnea (Neuroptera: Chrysopidae)*, 27 Environ. Entomol 1255-1263 (1998).

2. Hilbeck, A., M. Baumgartner, P.M. Fried, F. Bigler, *Effects of Bacillus thuringiensis corn-fed prey on mortality and development time of immature Chrysoperla carnea (Neuroptera: Chrysopidae)*, 27 (2) Environ. Entomol 480-487 (1998)

3. Hilbeck, *et al.*, 27 Environ. Entomol 1255-1263.

4. Hilbeck, *et al.*, 27 Environ. Entomol 1255-1263.

5. The first instars die as soon as they start eating *Bt* corn tissue.

6. ECB larvae live within the corn stalk, not on stalk surface.

7. EPA requires Tier IV field studies when toxicity due to *Bt* is observed in non target insect laboratory studies at field exposure rates. The December 1999 FIFRA SAP was consulted on whether EPA should require non target insect field studies for all plant-pesticide registrations.

8. Hilbeck, *et al.*, 27 Environ. Entomol 1255-1263.

9. Fitt, G.P., Martes, C.L. and Llewellyn, D.L., *Field evaluation and potential ecological impact of transgenic cottons in Australia*, 4 Biocontrol Sci.Tech. 535-549 (1994); Orr, D.B., and D.A. Landis, *Oviposition of European Corn Borer (Lepidoptera: Pyralidae) and Impact of natural Enemy Populations in Transgenic Versus Isogenic corn*, 90(4) J. Econ. Entomol. 905-909 (1997); Pilcher, C.D., M.E. Rice, J.J. Obrycki and L.C. Lewis, *Field and Laboratory Evaluations of Transgenic Bacillus thuringiensis Corn on Secondary lepidopteran pests (Lepidoptera: Noctuidae)*, 90(2) J. Econ. Entomol. 669-678 (1997).

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www.epa.gov/pesticides/biopesticides/factsheets/fs006458t.htm
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