United States Environmental Protection Agency

Office of Pesticide Programs

Biopesticide Fact Sheet

Bacillus thuringiensis Cry1Ab Delta-Endotoxin and the Genetic Material Necessary for Its Production in Corn [MON 810] (006430)

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

EPA Publication Number: EPA 730-F-00-006

I. DESCRIPTION OF THE PLANT PESTICIDE

Bacillus thuringiensis Cry1Ab Delta-Endotoxin and the Genetic Material Necessary for Its Production in Corn

OPP Chemical Code: 006430

Trade Name: Yieldgard®

Year of Initial Registration: 1996

Pesticide Type: Plant-Pesticide

U.S. and Foreign Producers:

Monsanto Company
700 Chesterfield Parkway North
St. Louis, MO 63198

II. USE SITES

Commercial Use in Field Corn.

III. REGISTRATIONS

On 5/29/96, BPPD registered *Bacillus thuringiensis* delta-endotoxin as produced by the *cry1Ab* gene and the genetic material for its production (PV-ZMCT01) in corn, EPA Reg. No. 524-492. Although this new active ingredient is not limited to a particular corn line, the registration was originally limited to corn line MON 801. On 7/16/96, BPPD amended this registration to allow plantings of corn line MON 810. Subsequent to the EPA granting the commercial use registration below, the MON 801 registration was voluntarily canceled by Monsanto (effective 5/8/98).

In 12/96, BPPD issued a new registration, EPA Reg. No. 524-489, which expanded the use of this plant-pesticide to include the commercial use for field corn for corn line MON 810 only with use limitations in the Southern cotton growing areas. This registration was amended in 8/98 and in 2/99 to allow increased use in the South. On 1/31/00, this registration was amended to implement amended insect resistance management refuges.

IV. SCIENCE ASSESSMENT

Monsanto's MON 810 Corn Line

Monsanto's corn line MON 810 was produced by ballistically transforming another proprietary corn line with plasmid construct PV-ZMCT01. Plasmid construct PV-ZMCT01 consists of plasmids PV-ZMBK07 & PV-ZMGT10 ballistically introduced together. The MON 810 only expresses a truncated version of Cry1Ab delta-endotoxin rather than the full length version of Cry1Ab, but the active site is still retained. MON 810 progeny do not express marker gene products in detectable levels.

A. Human Health

1. Product Analysis - Cry1Ab

Data were submitted which showed that the truncated Cry1Ab toxin extracted from corn leaf tissue displays characters and activities similar to that produced in *E. coli* modified to produce Cry1Ab. The similarities shown for the tryptic core proteins isolated from the plant and that produced in *E. coli* were identical molecular weights after SDS-PAGE, immunorecognition Western blots and ELISA, identical amino acid sequence for the N-terminus, lack of glycosylation and bioactivity against either European corn borer or corn earworm. This analysis supports the use of the microbially produced toxin as an analogue for the plant produced protein in mammalian toxicity testing.

2. Toxicology Assessment

There is a reasonable certainty that no harm will result from aggregate exposure to the United States population, including infants and children, to the Cry1Ab protein and the genetic material necessary for its production. This includes all anticipated dietary exposures, inhalation, and dermal exposures.

The data submitted regarding potential health effects of Cry1Ab include information on the biochemical characterization of the corn expressed protein, the acute oral toxicity of Cry1Ab, and *in vitro* digestibility studies of the protein. The results of these studies were determined applicable to evaluate human risk and the validity, completeness, and reliability of the available data from the studies were considered. The acute oral toxicity test of bacterially-derived Cry1Ab protein showed no test substance related deaths at a dose of 4000 mg/kg. This dose represents the highest amounts that could be administered with the microbially produced test substances.

Although Cry1Ab expression level data was required for an environmental fate and effects assessment, residue chemistry data were not required for a human health effects assessment of the subject plant-pesticide ingredients because of the lack of mammalian toxicity. Both (1) available information concerning the dietary consumption patterns of consumers (and major identifiable subgroups of consumers including infants and children) and (2) safety factors which, in the opinion of experts qualified by scientific training and experience to evaluate the safety of food additives, are generally recognized as appropriate for the use of animal experimentation data were not evaluated because the lack of mammalian toxicity at high levels of exposure demonstrate the safety of the product at levels above possible maximum exposure levels. This is similar to the Agency position regarding toxicity and the requirement of residue data for the microbial *Bacillus thuringiensis* products from which this plant-pesticide was derived. [See 40 CFR Sec. 158.740(b).] For microbial products, further toxicity testing to verify the observed effects and clarify the source of the effects (Tiers II & III) and residue data are triggered by significant acute effects in studies such as the mouse oral toxicity study.

The acute oral toxicity data submitted 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 effects were shown to be caused by the plant-pesticide, even at relatively high dose levels, the Cry1Ab delta-endotoxin protein is not considered toxic.

Adequate information was submitted to show that the Cry1Ab test material derived from microbial cultures were biochemically and functionally similar to the proteins produced by the plant-pesticide ingredients in corn. Production of microbially produced protein was chosen in order to obtain sufficient material for testing. In addition, the *in vitro* digestibility studies indicate the proteins would be rapidly degraded following ingestion.

The genetic material necessary for the production of the plant-pesticide active and inert ingredients are the nucleic acids (DNA) which comprise (1) genetic material encoding these proteins and (2) their regulatory regions. "Regulatory regions" are the genetic material that control the expression of the genetic material encoding the proteins, such as promoters, enhancers, and termination sequences. 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 as a component of food. 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 subject active and inert plant pesticidal ingredients. No mammalian toxicity has been reported since the product was registered.

EPA has considered available information on the variability of the sensitivities of major identifiable subgroups of consumers including infants and children and the neurological differences between infants and children and adults and the neurological differences between infants and children and adults and effects of *in utero* exposure to the plant-pesticides. Since Cry1Ab is a protein, allergenic sensitivities were considered. 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. Data has been submitted which demonstrates that the Cry1Ab delta-endotoxin is rapidly degraded by gastric fluid *in vitro* and is non-glycosylated. Studies submitted to EPA done in laboratory animals have not indicated any potential for allergic reactions to *B. thuringiensis* or its components, including the delta-endotoxin in the crystal protein. 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 despite significant oral, dermal and inhalation exposure to the microbial product. Several reports under FIFRA ' 6(a)2 have been made for various *Bacillus thuringiensis* products claiming allergic reactions. However, the Agency determined these reactions were not due to *Bacillus thuringiensis* itself or any of the Cry toxins. Thus, the potential for the Cry1Ab protein to be a food allergen is minimal.

EPA has considered available information on the cumulative effects of such residues and other substances that have a common mechanism of toxicity. These considerations included the cumulative effects on infants and children of such residues and other substances with a common mechanism of toxicity. Consideration of a common mode of toxicity is not appropriate given that there is no indication of mammalian toxicity of the plant-pesticides and no information that indicates that toxic effects would be cumulative with any other compounds.

EPA has considered available information on the aggregate exposure levels of consumers (and major identifiable subgroups of consumers) to the pesticide chemical residue and to other related substances. These considerations include dietary exposure under the tolerance exemption and all other tolerances or exemptions in effect for the plant-pesticide chemical residue, and exposure from non-occupational sources. Exposure via the skin or inhalation is not likely since the plant-pesticides are contained within plant cells which essentially eliminates these exposure routes or reduces these exposure routes to negligible. Oral exposure, at very low levels, may occur from ingestion of processed corn products and drinking water. However a lack of mammalian toxicity and the digestibility of the plant-pesticides has been demonstrated. The use sites for Cry1Ab delta endotoxin are all agricultural for control of lepidopteran insects. Therefore, exposure via residential or lawn use to infants and children is not expected.

EPA has considered available information on whether the plant-pesticides may have an effect in humans that is similar to an effect produced by a naturally occurring estrogen or other endocrine effects. The active ingredient is a protein plant-pesticide derived from the microorganism *Bacillus thuringiensis*. No known metabolite that acts as an "endocrine disrupter" is produced by this microorganism. Therefore, no adverse effects to the endocrine system is known or expected.

3. Tolerance Exemption Conclusions

The Agency has concluded that establishment of a tolerance is not necessary to protect the public health and established an exemption from tolerance requirements for the active ingredient in this product on 8/2/96 as set forth below. This exemption remains in effect pursuant to FFDCA section 408(j)(3). 40 CFR 180.1173 reads as follows:

> *Bacillus thuringiensis* Cry1Ab delta-endotoxin and the genetic material necessary for its production all plants. (*The tolerance exemption used the old nomenclature for Cry proteins. The final rule refers to CryIA(b). CryIA(b) is now Cry1Ab under the new nomenclature currently used by the scientific community.*)

Bacillus thuringiensis Cry1Ab delta-endotoxin and the genetic material necessary for its production in all plants are exempt from the requirement of a tolerance when used as plant-pesticides in all plant raw agricultural commodities. "Genetic material necessary for its production" means the genetic material 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 enhances.

B. Gene Flow Potential

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. Environmental Fate

1. Laboratory Degradation Study

B.t.k. Cry1Ab protein bioactivity, added to the soil as a component of corn line #754-10-1 tissue decreased with an estimated half life of 1.6 days and an estimated DT_{90} of 15 days.

Cry1Ab protein bioactivity of corn line #754-10-1 tissue incubated without soil decreased with an estimated half life of 25.6 days, and a DT_{90} of 40.7 days. The bioactivity of purified Cry1Ab protein in soil decreased with an estimated half life of 8.3 days and a DT_{90} of 32.5 days

2. Field Data

1994 field data regarding MON 810 demonstrated expression levels of 0.18-0.39 ug/g in grain, 7.93 -10.34 ug/g in the leaf, 3.65-4.65 ug/g in the whole plant, and 0.09 ug/g in the pollen. MON 810 does not express detectable levels of the marker gene products and the Cry1Ab protein is more truncated then in MON 801.

MON 810 was shown to be stable in expression between 1994 and 1995. 1995 U.S. field data showed 5.2-10.6 ug/g in the leaf, 2.3-4.5 ug/g in forage, and 0.4-0.9 ug/g in the grain. 1995 French field data showed 7.6-9.4 ug/g in the leaf, 4.1-5.6 ug/g in forage, and 0.4-0.7 ug/g in the kernel.

D. Ecological Effects

1. Background

Acceptable studies have been submitted which demonstrate that *E. coli*-derived, purified *B.t.k.* Cry1Ab toxin has minimal adverse impact on the honey bee, and other non-target insects (parasitic hymenopteran, green lacewing, and lady bird beetles), and soil organisms (earthworm). Quail and catfish studies were generated using Cry1Ab containing kernels. Additional data are needed to more fully characterize the risk to Collembola and aquatic invertebrates. However, the overall risk to a substantial number of individual non-target organisms in populations exposed to the levels of endotoxin found in plant tissue is anticipated

by the Agency to be minimal during the duration of this conditional registration.

MON 810 and MON 801 were each transformed with the same plasmid construct (PV-ZMCT01). The MON 810 progeny express a slightly truncated version of Cry1Ab compared to MON 801, but the active site is still retained. The MON 810 progeny do not express in detectable levels the marker gene products found in MON 801 progeny.

The level of Cry1Ab produced in corn line MON 801 progeny has decreased with breeding over time. On 5/29/96, BPPD registered *Bacillus thuringiensis* delta-endotoxin as produced by the *cry1Ab* gene and the genetic material necessary for its production (PV-ZMCT01) in corn. Although this new active ingredient is not limited to a particular corn line, the registration was originally limited to corn line MON 801.

On 7/16/96, BPPD amended this registration to allow plantings of corn line MON 810. However; additional studies of quail, catfish, and Daphnia were required for the full commercial registration of MON 810. These studies were listed as data gaps because although some of the data in the nontarget organism database supporting the registration were generated using *E. coli* produced *Bt* protein, the test substance for the quail and catfish studies already reviewed was MON 801 seed. Further, MON 810 expresses detectable levels of Cry1Ab in pollen and therefore may pose some degree of exposure to Daphnia, whereas MON 801 does not.

2. New Information

According to Monsanto "{t}he fish and quail studies were performed with MON 801 grain which expressed the Cry1Ab protein in the range of 0.2 - 0.9 ug/g fresh wt."

In response to the Agency's inquiry as to why there was such great variation for the MON 801 expression, Monsanto states the following in their 7/22/96 facsimile/email message: "The levels of CryI(b) protein in leaves, grain and whole plants of MON 801 have decreased during breeding. The reason for the decrease is not known. The DNA insert in line MON 801 is stable, as demonstrated through Southern blot analysis. The decrease in expression appears to be related to the repeated cycles of inbreeding required to convert the inbred parents. Since the breeding started in 1992, the expression has not increased in any of the approximately 150 hybrids tested to date. There has been no published evidence of transgene expression increasing during breeding." No such decrease has been observed with MON 810 and is therefore not anticipated.

3. MON 801 Data Applicability to MON 810 Progeny

Given the dosing and expression level information now available to the Agency, the MON 801 quail and catfish data are applicable to the MON 810 line progeny since the levels of Cry1Ab are similar. The MON 801 line of corn is similar to MON 810 corn in that they both were derived from transformation events utilizing PV-ZMCT01.

4. Impacts on Non-Target Organisms

a. Impacts on Non-Target Insect - Honey Bee (Larvae)

No adverse effects were observed at a maximum hazard dose of 20 ppm *B.t.k*. HD-1 protein. An LC_{50} was not possible to calculate since this was a single dose test. Therefore, the NOEL is greater than 20 ppm.

b. Impacts on Non-Target Insect - Honey Bee (Adult)

There were no statistically significant differences among the various treatment and control groups due to the sizable mortality that occurred in all treatments. B.t.k. HD-1 protein at 20 ppm resulted in a mean mortality of 16.2%. Because mortality was observed at the single dose tested, a NOEL could not be determined from this study, but it was less than 20 ppm. 20 ppm was determined to be significantly higher than exposure conditions in the environment.

c. Impacts on Non-Target Insect - Parasitic Hymenopteran

No adverse effects were observed at a maximum hazard dose of 20 ppm B.t.k. HD-1 protein to *Brachymeria intermedia*. Since this is a single dose study, an LC_{50} cannot be calculated. The NOEL is greater than 20ppm.

d. Impacts on Non-target Insect - Green Lacewing Larvae

No adverse effects were observed at a maximum hazard dose of 16.7 ppm B.t.k. HD-1 protein after 7 days. The NOEL is greater than 16.7 ppm.

e. Impacts on Nontarget Insect - Lady Beetles

No adverse effects were observed at a maximum hazard dose of 20 ppm B.t.k. HD-1 protein. The NOEL is greater than 20 ppm.

f. Impacts on Birds - Northern Bobwhite Quail

No treatment related mortality or differences in food consumption, body weight or behavior occurred in birds fed 50,000 or 100,000 ppm transgenic corn meal derived from Monsanto's MON 80187 corn line (which contains Cry1Ab protein) relative to birds fed corn meal made from parental corn lines which did not express *Bt* toxin.

Although this study utilized Monsanto's MON 801 *Bt* corn for testing, the test material was considered sufficiently similar to the MON 810 corn grain to bridge the data because of the similarity in Cry1Ab levels.

g. Impacts on Earthworm

The 14-Day LC_{50} value for earthworms exposed to Cry1Ab insecticidal protein derived from *E. coli* in an artificial soil substrate was determined to be greater than 200 mg/kg (ppm), which was the single concentration tested. There were no statistically significant effects at the single dose tested. Therefore, the NOEL is greater than 200 ppm. Although this study was graded supplemental, *Bt* toxins expressed in the corn plant are not expected to generate a toxic effect in the earthworm; therefore, no additional follow-up of this study is required.

h. Impacts on Collembola

Impacts on non-target soil organisms are of interest because of the residual B.t.k. protein that exists in the corn plant at physiological maturity and the potential for incorporation into the soil. Monsanto has submitted a study assessing impacts on *Collembola spp*., which has been rated as a "supplemental" study due to the form of the test material. The Agency asked for a *Collembola* study using lyophilized leaf extract as the test material subsequent to the initial registration application, but, to date, the registrant has only cited one using purified Cry1Ab toxin derived from *E. coli* as the test substance. Therefore, Monsanto must fulfill this unfulfilled data requirement and submit or cite the required Collembola study. [Monsanto has subsequently submitted the *Collembola* study using lyophilized leaf extract, however, the Agency has not yet completed its review of the study.]

In the study submitted by Monsanto, purified B.t.k. insecticidal proteins derived from *E. coli* (200 ppm), including Cry1Ab toxin, had no observable toxicological effect on two species of Collembola: *Folsomia candida* and *Xenylla grisea.* The applicant has been informed via Agency letter that this study does not adequately addresses the Agency's non-target soil organism questions because it was conducted with purified *E. coli*-produced B.t.k. protein and not lyophilized leaf extract, as the Agency requested. The rationale for the required study is that there is another study on file that demonstrates toxicity to Collembola, using lyophilized leaf extract as the test material, while control leaf extract did not.

i. Impacts on Channel Catfish

The study "Evaluation of the European Corn Borer Resistant Corn Line MON 801 as a Feed Ingredient for Catfish" was reviewed to determine potential impacts on channel catfish from Monsanto's MON 810 corn lines. Feed per fish, feed conversion ratios, final weight, percentage weight gain and survival were not significantly different between fish fed the control MON 800 diet when compared to those fed the diet containing transgenic corn from the test line MON 801. Body composition data exhibited no significant differences in percentage moisture, fat, or ash, with a higher protein content in the test fish on a dry weight basis. This difference in protein content disappears when one expresses the results on a wet weight basis. Data in this study are consistent with historical controls for catfish grown at the Delta Research and Extension Center.

Although this study utilized Monsanto's *MON 801 Bt* corn for testing, the test material was considered sufficiently similar to the MON 810 corn grain to bridge the results for the data requirement since the levels of Cry1Ab in the MON 801 grain tested were similar to MON 810 levels.

j. Impacts on Aquatic Invertebrates

Due to the potential exposure of aquatic invertebrates to corn pollen containing the *Bt* Cry1Ab toxin, this requirement will need to be addressed by the applicant by conducting a *Daphnia magna* study; or by providing adequate rationale for waiver. *[Monsanto has subsequently submitted a Daphnia magna study, however, the Agency has not yet completed its review of the study.]*

k. Impacts on Mammals

Both the scientific literature and the acute oral mouse study results indicate that no toxicity is expected in mammals. Therefore, no further testing on mammals in indicated

l. Impacts on Non-Target Lepidopterans and Endangered Species

In the *Bacillus thuringiensis* (*Bt*) Reregistration Eligibility Decision document (RED)*[PDF]*, 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 Monsanto 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.

E. 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.

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.

In 1997, Monsanto (Cry1Ab) required growers to sign a grower contract that mandated that growers plant either a 5% unsprayed non-*Bt* corn refuge or a 20% sprayable non-*Bt* corn refuge.

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).

Also, in 1998, EPA approved the registration of Novartis' Cry1Ab (BT11) sweet corn. EPA mandated specific resistance monitoring requirements for this registration for ECB, CEW, and fall armyworm *(Spodoptera frugiperda* (J.E. Smith)), as well as sales reporting requirements. Specific refuge requirements were not mandated for this *Bt* sweet corn product because sweet corn harvesting occurs before insects mature. Novartis is required through labeling and technical material to have growers destroy any Cry1Ab sweet corn stalks that remain in the fields following harvest in accordance with local production practices. Stalk destruction is intended to reduce the possibility of any insects, including resistant insects, surviving to the next generation.

For the 1999 growing season, EPA required that Monsanto mandate (through grower contracts) a 10% unsprayed or 20% sprayed refuge within close proximity of *Bt* corn fields in the Corn Belt for its Cry1Ab field corn.

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 ¼ mile of their *Bt* corn fields.

In addition, for the 2000 growing season, specific regional monitoring plans must be expanded to include ECB, SWCB, and CEW. 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.

VI. TERMS AND CONDITIONS OF THE REGISTRATION

The following listing gives the current terms and conditions of the registration.

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

B. This registration is for field corn only.

C. 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.

D. 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, Linclon, 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).

E. 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.

F. Monsanto will monitor for the development of resistance using baseline susceptibility data and/or a discriminating concentration assay when such an assay is available. Monsanto will proceed with efforts to develop a discriminating concentration assay. Monsanto will ensure that monitoring studies are conducted annually to determine the susceptibility of ECB and corn earworm (CEW) populations to the Cry1Ab protein. This resistance monitoring program will be developed to measure increased tolerance to *Bt* corn above the various regional baseline ranges.

Populations of ECB and CEW will be collected from representative distribution areas that contain Monsanto's *Bt* corn plant-pesticide and monitored/screened for resistance, with particular focus on those areas of highest distribution. The results of 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, Monsanto will instruct its customers (growers and seed distributors) to contact Monsanto (e.g., via a toll-free customer service number) if incidents of unexpected levels of ECB and/or CEW damage occur. Monsanto will investigate and identify the cause for this damage by local field sampling of plant tissue from corn hybrids that contain Monsanto's *Bt* corn plant-pesticide and sampling of ECB & CEW populations, followed by appropriate *in vitro* and *in planta* assays. Upon Monsanto'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 by Monsanto, Monsanto will utilize the following to define a confirmed instance of ECB and/or CEW resistance:

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

1. 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 or CEW 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.

2. > 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 Monsanto finds it appropriate to alter the criteria specified in the working definition, Monsanto must obtain Agency approval in establishing a more suitable definition.

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.]*

G. 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.

H. When resistance has been demonstrated to have occurred, you 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, Monsanto assumes 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.

I. Monsanto will maintain a (confidential) database to track sales (units and location) of its *Bt* corn on a county-by-county basis. Monsanto will provide annually, on a CBI basis, sales data for each state indicating the number of units of corn hybrids that contain Monsanto's *Bt* corn plant-pesticide that were sold. As part of the overall sales report, Monsanto will provide a listing of an estimate of the acreage planted with such states and counties with sales limitations. This information will be provided by January 31 of the year following each growing season.

J. Monsanto will provide grower education. Monsanto will agree to include an active partnership with such parties as: university extension entomologists and agronomists, consultants, and corn grower groups. Monsanto will implement a grower education program (in part, as requested by Monsanto, through the Grower Agreement setting forth any resistance management requirements) directed at increasing grower awareness of resistance management, in order to promote responsible product use. Insect Resistance Management 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."

K. Several aspects of the Insect Resistance Management Plan will operate in synergy to promote grower compliance, however, the cornerstones of the compliance program must be the:

> 1. 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.
>
> 2. 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.
>
> 3. 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.
>
> 4. 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).

L. Monsanto will confer with the EPA as Monsanto develops various aspects of its resistance management research program. Monsanto agrees, as a condition of this registration, to submit annually progress reports on or before January 31st each year on the following areas as a basis for developing a long-term resistance management strategy which include:

> 1. Monsanto must submit by January 31, 1997, available research data on CEW relative to resistance development and Monsanto's plans for producing resistance predictive models to cover regional management zones in the cotton belt based on *Helicoverpa zea* biology and cotton, corn, soybeans, and other host plants. [These data have been submitted, reviewed, and satisfied thus far.] These models must be field tested and must be modified based on the field testing performed during the period of the conditional registration. EPA might

modify the terms of the conditional registration based upon the field testing validation of the model and might require refuge in the future. EPA notes that there is some scientific work and even some models for H. zea on other crops in at least NC and TX that could be used for reference. EPA wants to be in close communication with Monsanto as the model development and testing is ongoing. The requirement for development of resistance predictive models may be modified if Monsanto provides the results of research that demonstrates resistance to CEW would have no significant impact on the efficacy of foliar *Bt* products and other *Bt* crops. Actual usage data of Btk on crops to control specific pests as well as successes and failures and field validated research would be necessary to support such a waiver request.

2. 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. A combination of a comprehensive literature review and research can fulfill this condition.

3. The feasibility of "structured" refuge options for ECB including both "block" refuge, "50-50 early/late season patchwork;" research needs to be done in both northern and southern areas on ECB as well as CEW.

4. Development of a discriminating concentration (diagnostic concentration) assay for field resistance (field screening) for ECB, CEW and other Lepidoptera pests of corn. Specific sampling locations will be established in each state to determine if increases in *Bt* toxin tolerance are occurring before crop failures develop. Increased tolerance levels need to be identified before field failure occurs. In monitoring for tunneling damage, the number of trivial tunnels may be less indicative of resistance development than the total extent of tunneling damage (e.g. length of tunnels). The extent of tunneling damage must be monitored as well as the number of tunnels.

5. Effects of corn producing the Cry1Ab delta endotoxin on pests other than ECB, including but not limited to CEW, fall armyworm, and the stalk borer complex.

6. 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. Possible high dose control exists for the first generation ECB in whorl stage, but not for later generation(s) on more mature corn plants. More data are needed on toxin expression in various parts of the plant at different stages plant development in regard to ECB, CEW and other secondary pests of corn (i.e. stalk borer complex, fall armyworm, and SW corn borer).

7. You 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.

8. You 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:

a) What is your primary source of information on *Bt* corn?

b) What percentage of your acres were planted to *Bt* corn this year?

c) Are you following a recommended insect resistance management strategy?

d) 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?

e) Did you treat your *Bt* corn acres with an insecticide?

f) 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.

M. *Collembola* and *Daphnia magna* studies must be submitted by 5/14/97 for this active ingredient. *[These studies have been submitted and are under EPA review]*

VII. CONTACT PERSON AT EPA

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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.*

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www.epa.gov/pesticides/biopesticides/factsheets/fs006430t.htm
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