United States
Environmental Protection
Agency

Office of Prevention,
Pesticides
and Toxic Substances
(7501C)

PESTICIDE FACT SHEET

Name of Chemical(s): *Bacillus thuringiensis* subspecies Cry1F Protein and the Genetic Material Necessary for Its Production (Plasmid Insert PHI 8999) in Corn

Reason for Issuance: New Registration

Date Issued: July 2001

EPA Publication Number:

I. DESCRIPTION OF THE PLANT PESTICIDE

Bacillus thuringiensis subspecies Cry1F Protein and the Genetic Material Necessary for Its Production (Plasmid Insert PHI 8999) in Corn

OPP Chemical Code: 006481

Trade Name: Herculex™ I Insect Protection, Pioneer Brand Seed Corn with Herculex™ I

Year of Initial Registration: 2001

Pesticide Type: Plant-Pesticide

U.S. and Foreign Producers:

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

Pioneer Hi-Bred International, Inc.
7250 NW 62nd Avenue
P.O. Box 552
Johnston, Iowa 50131-0552

II. USE SITES AND LIMITATIONS

Bacillus thuringiensis subspecies Cry1F protein and the genetic material necessary for its production (plasmid insert PHI 8999) in corn is registered for full commercial use in field corn originating from maize line 1507 until September 30, 2001.

III. BT CROP REASSESSMENT PROCESS

In order to link these Cry1F Bt corn registrations to the current Bt crops reassessment process that the Agency is undergoing to ensure that any new necessary modifications to the registration and data requirements that are determined for Bt crops during the reassessment are imposed for these products, an expiration date of September 30, 2001 for the Cry1F products was imposed to match the expiration date of the currently registered Bt corn products being evaluated in the reassessment.

EPA is currently engaged in a comprehensive reassessment of the time-limited registrations for all existing B.t. corn and cotton plant-pesticides. This reassessment has been designed to assure that the decisions on the renewal of these registrations are based on the most current health and ecological data. Current registrations are set to expire September 30, 2001. As part of EPA's reassessment, the Agency will be decide whether to extend the registrations and whether to include any additional terms and conditions of such registrations for issues including insect resistance management, the protection of non-target organisms, and other measures necessary to ensure full public and environmental safety.

IV. SCIENCE ASSESSMENT

A. Product Analysis

1. Product Analysis - Cry1F

A modified (synthetic, less than full-length) form of the *cry*1Fa2 gene and the phosphinothricin acetyl transferase (*pat*) gene were inserted into maize plants by microprojectile bombardment. Digestion of the genomic DNA of maize line 1507 with *Nhe*I or *Hind*III and Southern hybridization with probes specific for *cry1F*, *kan*^{r} and *pat* genes yielded indications of the complexity of the gene integration pattern and copy number. Hybridization patterns suggested that the copy number of introduced / integrated *cry1F* and *pat* genes is one. It is most likely that the TC 1507 line contains one functional *cry1F* gene and partial copies (1 or 2) of the gene which are non-functional.

B. Human Health Assessment

1. Mammalian Toxicity and Allergenicity Assessment

Data have been submitted demonstrating the lack of mammalian toxicity at high levels of exposure to the pure Cry1F protein. These data demonstrate the safety of the products at levels well above maximum possible exposure levels that are reasonably anticipated in the crops. 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)(2)(i).] For microbial products, further toxicity testing and residue data are triggered by significant acute effects in studies such as the mouse oral toxicity study, to verify the observed effects and clarify the source of these effects (Tiers II & III).

The acute oral toxicity data submitted support the prediction that the Cry1F protein would be non-toxic to humans. Male and female mice (5 of each) were dosed with 15 % (w/v) of the test substance, which consisted of *Bacillus thuringiensis* var. *aizawai* Cry1F protein at a net concentration of 11.4 %. Two doses were administered approximately an hour apart to achieve the dose totaling 33.7 mL / kg body weight. Outward clinical signs and body weights were observed and recorded throughout the 14 day study. Gross necropsies performed at the end of the study indicated no findings of toxicity. No mortality or clinical signs were noted during the study. An LD_{50} was estimated at >5050 mg / kg body weight of this microbially produced test material. The actual dose administered contained 576 mg Cry1F protein / kg body weight. At this dose, no LD_{50} was demonstrated as no toxicity was observed. Cry1F maize seeds contain 0.0017 to 0.0034 mg of Cry1F / gram of corn kernel tissue.

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-pesticides, even at relatively high dose levels, the Cry1F protein is not considered toxic. Further, amino acid sequence comparisons showed no similarity between Cry1F protein to known toxic proteins available in public protein databases.

Since Cry1F 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, may be glycosylated and present at high concentrations in the food.

Data has been submitted which demonstrates that the Cry1F protein is rapidly degraded by gastric fluid *in vitro* and is non-glycosylated. In a solution of Cry1F:pepsin at a molar ratio of 1:100, complete degradation of Cry1F to amino acids and small peptides occurred in 5 minutes. A heat lability study demonstrated the loss of bioactivity of Cry1F protein to neonate tobacco budworm larvae after 30 minutes at 75 °C. Studies submitted to EPA done in laboratory animals have not indicated any potential for allergic reactions to *B. thuringiensis* or its components, including the δ-endotoxin of the crystal protein. Additionally, a comparison of amino acid sequences of known allergens uncovered no evidence of any homology with Cry1F, even at the level of 8 contiguous amino acids residues.

The potential for the Cry1F protein to be a food allergen is minimal. Regarding toxicity to the

immune system, the acute oral toxicity data submitted support the prediction that the Cry1F 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-pesticides, even at relatively high dose levels, the Cry1F protein is not considered toxic.]

2. Aggregate Exposures

Pursuant to FFDCA section 408(b)(2)(D)(vi), EPA considers available information concerning aggregate exposures from the pesticide residue in food and all other non-occupational exposures, including drinking water from ground water or surface water and exposure through pesticide use in gardens, lawns, or buildings (residential and other indoor uses).

The Agency 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-pesticide is 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, potentially, drinking water. However a lack of mammalian toxicity and the digestibility of the plant-pesticides have been demonstrated. The use sites for the Cry1F protein are all agricultural for control of insects. Therefore, exposure via residential or lawn use to infants and children is not expected. Even if negligible exposure should occur, the Agency concludes that such exposure would present no risk due to the lack of toxicity demonstrated for the Cry1F protein.

3. Cumulative Effects

Pursuant to FFDCA Section 408(b)(2)(D)(v), 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. Because there is no indication of mammalian toxicity to these plant-pesticides, we conclude that there are no cumulative effects for the Cry1F protein.

4. Determination of Safety for U.S. Population, Infants and Children

a) Toxicity and Allergenicity Conclusions

The data submitted and cited regarding potential health effects for the Cry1F protein include the characterization of the expressed Cry1F protein in corn, as well as the acute oral toxicity, heat stability, and *in vitro* digestibility of the proteins. 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.

Adequate information was submitted to show that the Cry1F test material derived from microbial cultures was biochemically and, functionally similar to the protein produced by the plant-pesticide ingredients in corn. Production of microbially produced protein was chosen in order to obtain sufficient material for testing.

The acute oral toxicity data submitted supports the prediction that the Cry1F 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)]. Since no effects were shown to be caused by Cry1F protein, even at relatively high dose levels (>5,050 mg test substance / kg body weight; 576 mg Cry1F / kg body weight), the Cry1F protein is not considered 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)(2)(i).] For microbial products, further toxicity testing and residue data are triggered by significant acute effects in studies such as the mouse oral toxicity study to verify the observed effects and clarify the source of these effects (Tiers II & III).

Although Cry1F 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. The lack of mammalian toxicity at high levels of exposure to the Cry1F protein demonstrates the safety of the product at levels well above possible maximum exposure levels anticipated in the crop.

The genetic material necessary for the production of the plant-pesticides active ingredients are the nucleic acids (DNA, RNA) which comprise (1) genetic material encoding these proteins and (2) their regulatory regions. "Regulatory regions" are the genetic material, such as promoters, terminators, and enhancers, that control the expression of the genetic material encoding the proteins. DNA and RNA are 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 plant pesticidal ingredients.

b) Infants and Children Risk Conclusions

FFDCA section 408(b)(2)(C) provides that EPA shall assess the available information about consumption patterns among infants and children, special susceptibility of infants and children to pesticide chemical residues and the cumulative effects on infants and children of the residues and other substances with a common mechanism of toxicity. In addition, FFDCA section 408(B)(2)(C) also provides that EPA shall apply an additional tenfold margin of safety for infants and children in the case of threshold effects to account for pre- and post-natal toxicity and the completeness of the database unless EPA determines that a different margin of safety will be safe for infants and children.

In this instance, based on all the available information, the Agency concludes that there is a finding of no toxicity for the Cry1F protein and the genetic material necessary for its production. Thus, there are no threshold effects of concern and, as a result, the provision requiring an additional margin of safety does not apply. Further, the provisions of consumption patterns, special susceptibility, and cumulative effects do not apply.

c) Overall Safety Conclusion

There is a reasonable certainty that no harm will result from aggregate exposure to the U.S. population, including infants and children, to the Cry1F protein and the genetic material necessary for its production. This includes all anticipated dietary exposures and all other exposures for which there is reliable information.

The Agency has arrived at this conclusion because, as discussed above, no toxicity to mammals has been observed for the plant-pesticides.

5. Other Considerations

a) Endocrine Disruptors

The pesticidal active ingredients are proteins, derived from sources that are not known to exert an influence on the endocrine system. Therefore, the Agency is not requiring information on the endocrine effects of these plant-pesticides at this time.

b) Analytical Method(s)

A validated method for extraction and direct ELISA analysis of Cry1F in corn grain has been submitted and found acceptable by the Agency.

c) Codex Maximum Residue Level

No Codex maximum residue levels exists for the plant-pesticides *Bacillus thuringiensis* Cry1F

protein and the genetic material necessary for its production in corn.

6. Tolerance Exemption

Therefore, 40 CFR chapter I is to be amended as follows:

Bacillus thuringiensis Cry1F Protein and the Genetic Material Necessary for its Production in Corn.

Bacillus thuringiensis Cry1F protein and the genetic material necessary for its production in corn are exempt from the requirement of a tolerance when used as plant-pesticides in the food and feed commodities of field corn, sweet corn and popcorn. ``Genetic material necessary for its production'' means the genetic material which comprise (1) genetic material encoding the Cry1F protein and (2) its regulatory regions. ``Regulatory regions'' are the genetic material, such as promoters, terminators, and enhancers, that control the expression of the genetic material encoding the Cry1F protein.

C. Environmental Assessment

1. Ecological Effects Hazard Assessment

This environment hazard assessment includes outcrossing and potential for weeds to develop if pollen from Cry1F corn was to fertilize other plants, horizontal gene transfer, expression of Cry1F protein in plant tissues, ecological effects including effects on monarch butterflies, fate of Bt proteins in the environment and effects on endangered species, particularly Lepidoptera. Studies have been submitted which demonstrate no effects under test conditions to representative species of birds (Bobwhite quail), non-target soil organisms (*Collembola* and Earthworm), honey bees, ladybird beetle, green lacewing, parasitic wasp, the monarch butterfly, aquatic invertebrates (*Daphnia magna*) and non-target insects in corn fields. In addition, it has been shown that conventional processes used in the commercial preparation of fish food inactivate any Cry1F protein present in corn grain. Cry1F protein in soil has been shown to degrade rapidly to very low levels.

2. Outcrossing and Weediness

The movement of transgenes from the host plant into weeds and other crops has been a significant concern due to the possibility of novel exposures to the pesticidal substance. The Agency has determined that there is no significant risk of gene capture and expression of Cry1F protein by wild or weedy relatives of corn in the U.S., its possessions or territories. Domesticated corn does not have a reasonable possibility of passing its traits to wild maize species. Feral species related to corn, as found within the United States, cannot be pollinated due to differences in chromosome number, phenology (periodicity or timing of events within an organism’s life cycle as related to climate, e.g., flowering time) and habitat.

However, concern over species related to maize (*Zea mays* ssp. *mays*), such as *Tripsacum* species and the teosintes, as potential recipients of gene flow from genetically modified *Zea mays* calls for a closer look at this topic. Some *Zea* spp., such as the teosintes, are known to be interfertile with maize and are discussed as potential recipients of pollen directed gene flow from maize. This issue is of particular concern based upon the increased planting of genetically modified maize.

a) *Zea mays* ssp. *mays* - Maize

The origin of corn is thought to be in Mexico or Central America, based largely on archaeological evidence of early cob-like maize in indigenous cultures approximately 7200 years ago. *Zea mays* is a wind-pollinated, monoecious, annual species with imperfect flowers. This means that spatially separate tassels (male flowers) and silks (female flowers) are found on the same plant, a feature which limits inbreeding. A large variety of types are known to exist (*e.g.*, dent, flint, flour, pop, sweet) and have been selected for specific seed characteristics through standard breeding techniques. Maize cultivars and landraces are known to be diploid (2n = 20) and interfertile to a large degree. However, some evidence for genetic incompatibility exists within the species (*e.g.*, popcorn x dent crosses; Mexican maize landraces x Chalco teosinte). *Zea mays* has been domesticated for its current use by selection of key agronomic characters, such as a non-shattering rachis, grain yield and resistance to pests.

A recent study has indicated that cross-pollination of commercial maize cultivars at 100 ft downwind 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 160 ft. At 1000 ft, the farthest distance measured, no cross-pollination was detected. For production of Foundation Seed, a distance of 660 ft has been generally required to ensure separation of pollen types. The relatively large size of corn pollen and its short viability period under most conditions preclude long distance transfer for purposes of outcrossing. Under conditions of high temperature or low humidity, corn pollen may only survive for a matter of minutes. Under more favorable conditions in the field or with controlled handling in the laboratory, pollen life may be extended to several hours.

b) *Tripsacum* species - Gama Grass

A close relative of corn or maize is the genus *Tripsacum.* 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.. In the Manual of Grasses of the United States, A. S. Hitchcock (revisions by Agnes Chase; 1971) 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 known

from 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 native to 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); and *T. lanceolatum* (2n = 72) which occurs in the Southwestern U.S. *Tripsacum* differs from corn in many respects, including chromosome number (*T. dactyloides* 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. *Tripsacum* / maize hybrids have not been observed in the field, but have been accomplished in the laboratory using special techniques under highly controlled conditions.

Eastern Gama Grass 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 (maize n = 10; Gama Grass x = 9 or 18, with diploid, triploid and tetraploid races existing; 2n = 36 or 72), 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* 'embryo rescue' techniques.

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. Approximately 10 - 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 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.

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. In many instances where hybridization has been directed between these two species, the resultant genome is lacking in most or all of the chromosomal complements of one of the parent species in subsequent generations.

Conclusion: The possibility of maize contributing genetic material to Eastern Gama Grass

through random pollen flow in agricultural or natural situations is extremely remote based upon experience trying to create hybrids under the optimal laboratory conditions. No other known grass species present in the continental U.S. would interbreed with commercial maize populations (*i.e.*, be recipients of pollen-directed gene flow). None of the sexually compatible relatives of corn in the U.S. are considered to be serious, principal, or common weeds in the U.S.

c) *Zea* species - Teosintes

Teosintes, specifically *Z. mays* ssp. *mexicana* (Schrader) Iltis, *Z. mays* ssp. *parviglumis* Iltis and Doebley, *Z. mays* ssp. *huehuetenangensis* (Iltis and Doebley) Doebley, *Z. luxurians* (Durieu and Ascherson) Bird, *Z. perennis* (Hitchc.) Reeves and Mangelsdorf and *Z. diploperennis* Iltis, Doebley and Guzman, have co-existed and co-evolved in close proximity to maize in the Americas over thousands of years, however, maize and teosinte maintain distinct genetic constitutions despite sporadic introgression.

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 could 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.

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, especially when maize acts as the female parent. *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. A few isolated populations of annual and perennial teosinte were said to exist in Florida and Texas, respectively. The Florida populations were presumably an escape from previous use of *Z. mays*

ssp. *mexicana* as a forage grass, but local botanists have not documented any natural populations of this species for approximately twenty-five years. No teosinte populations are reported to exist in the State of Texas. Further, given the day length characteristics of *Z. diploperennis*, it is highly unlikely a sustaining population would result from introduction of this species. *Z. mays* ssp. *mexicana, Z. mays* ssp. *parviglumis, Z. luxurians* and *Z. diploperennis* may cross with maize to produce fertile hybrids in many instances. None of these teosinte species have, however, been shown to be aggressive weeds in their native or introduced habitats. 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, El Salvador and Guatemala.

Given the cultural and biological relationships of various teosinte species and cultivated maize over the previous two millennia, it would appear that significant gene exchange has occurred (based upon morphological characters) between these two groups of plants and that no weedy types have successfully evolved as a result. More recent cytogenetic, biochemical and molecular analyses have indicated that the degree of gene exchange is far less than previously thought. Partial and complete gametophytic incompatibility has been documented among cultivated maize, landraces and teosinte . The former is demonstrated by differential pollen growth and a skewed recovery of alleles linked to incompatibility genes. Complete incompatibility mechanisms serve to isolate a species or subspecies and are evidenced as pollen exclusion or non-functioning of pollen types on certain genotypes. Attempts to cross six collections of *Zea mays* ssp. *mexicana* with U.S. maize cultivars (W22, W23) yielded no or few seeds in five of the six groups.

Conclusion: Based on the ability of maize to hybridize with some teosintes, the suggestion of previous genetic exchange amongst these species over centuries, 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.

Summary:

The potential for pollen-directed gene flow from maize to Eastern Gama Grass is extremely remote. This is evidenced by the difficulty with which *Tripsacum dactyloides* x *Zea mays* hybrids are produced in structured breeding programs. Additionally, the genus does not represent any species considered as serious or pernicious weeds in the United States or its territories. Any introgression of genes into this species as a result of cross fertilization with genetically-modified maize is not expected to result in a species that is weedy or difficult to control. In many instances where hybridization has been directed between these two species, the resultant genome is lacking in most or all of the maize chromosomal complement in subsequent generations.

Many of the *Zea* species loosely referred to as “teosintes” will produce viable offspring when crossed with *Zea mays* ssp. *mays*. None of these plants are known to harbor weedy characteristics and none of the native teosinte species, subspecies or races are considered to be aggressive weeds in their native or introduced habitats. In fact, many are on the brink of

extinction where they are indigenous and will be lost without human intervention (*i.e.*, conservation measures). Further, none of the landraces or cultivated lines of *Zea mays* are considered to have weedy potential and are generally considered to be incapable of survival in the wild as a result of breeding practices (*i.e.*, selection) during domestication of the crop.

3. Ecological Exposure and Risk Characterization

a. Ecological Exposure

1) Maximum Expression of Cry1F Protein in Various Corn Tissues

Cry1F protein from inbred and hybrid maize 1507 pollen, grain, grain-derived feeds and a microbial source was evaluated biochemically using ELISA, SDS-PAGE and Western Blotting, and for bioactivity using insect bioassays. Transgene expression was found throughout the different plant tissues across the growing season. The level of the Cry1F proteins was higher in tissues and in whole plants during vegetative growth through pollen shed and declined with plant senescence. PAT expression was found to be typically below the detection limit.

a) Cry1F and PAT protein expression in hybrid maize samples:

Test line grain samples contained an average Cry1F expression of 89.8 (71.2 to 114.8) pg / µg total protein. Leaf sample expression from Cry1F maize lines was 110.9 (56.6 to 148.9) pg / µg total protein. Pollen and silk samples yielded 135.5 (113.4 to 168.2) pg/µg total protein for pollen (31 to 33 ng/mg pollen) and 50.3 (26.8 to 79.8) pg / µg total protein for silk. The Cry1F expression for stalk samples was 550.0 (355.9 to 737.4) pg / µg total protein. For whole plant samples, the expression level averaged 1063.8 (803.2 to 1572.7) pg / µg total protein. In senescent whole plant samples the expression of Cry1F was 714.3 (622.2 to 845.3) pg / µg total protein. Of the leaf samples tested for PAT expression, the test line samples ranged from below the LOD to 40.8 pg / µg total protein. All of the following tissues were below the LOD for PAT: pollen, silk, stalk and grain from both test and control lines. Both whole plant samples and senescent whole plant samples were negative or below the LOD for PAT.

b) Cry1F and PAT protein expression in inbred maize samples:

Test line grain samples contained an average Cry1F expression of 112.2 (66.5 to 141.5) pg / µg total protein. Leaf sample expression from Cry1F maize lines was 169.5 (79.3 to 209.4) pg / µg total protein. Pollen and silk samples yielded 207.5 (186.3 to 231.1) pg/µg total protein for pollen and 58.9 (36.2 to 89.8) pg / µg total protein for silk. The Cry1F expression for stalk samples was 637.8 (480.5 to 849.0) pg / µg total protein. For whole plant samples, the expression level averaged 1357.8 (1283.5 to 1428.0) pg / µg total protein. In senescent whole plant samples the expression of Cry1F was 677.5 (470.5 to 968.3) pg / µg total protein. Of the leaf samples tested for PAT expression, the test line samples ranged from below the LOD to 58.2

pg / µg total protein. All of the following tissues were below the LOD for PAT: pollen, silk, stalk and grain from both test and control lines. Both whole plant samples and senescent whole plant samples were negative or below the LOD for PAT.

2) Half-Life and Estimated Environmental Concentration

Based on a bioassay with the tobacco budworm (*Heliothis virescens*), a target species, purified Cry1F proteins incorporated into test soils biodegraded with a half-life of approximately 3.13 days (Table 2). This half-life is very comparable with the 4-7 days in published reports for other Cry proteins. The study does not, however, adequately address the duration and the amount of residual Cry 1F protein in the soil.

Much of the Cry1F that will be exposed to the soil or soil organisms in the field consists of the protein in various corn tissues, e.g. incorporation of crop debris at the end of the growing season, pollen, or root tissue. Several published studies indicate that Cry proteins expressed in transgenic corn degrade more rapidly in the soil than purified Cry protein. Testing of purified protein degradation in the soil, therefore, may result in higher soil half-life than the degradation of plant incorporated Cry1F. Therefore addition of purified Cry protein is likely a more rigorous test of degradation rates than addition of Cry1F corn tissue. The reported 3.13 day half life of purified protein does, however, indicate that the Cry1F protein will be degraded rapidly in the soil to levels below those that could pose a hazard to non-target organisms.

RECOMMENDATIONS: There is no evidence to indicate that prolonged exposure to trace amounts of Cry protein in the soil affects non-target organisms. The submitted data do not, however, sufficiently address the issue of residual Cry protein accumulation in the soil. The soil degradation study should be carried out for a longer period of time to determine the duration and the amount of residual Cry 1F protein in agricultural soil. Also, the soil used in the study should be actual field soil containing the microbial flora normally found in the field. This will give a more accurate rate of degradation of the Cry protein in the agricultural environment because microbial populations in the rhizosphere are commonly 100 fold higher than in bulk soil. Bulk soil generally does not support populations of microorganisms as high as those in the rhizosphere or those in soils with high organic content (plant residues). In addition, field soil high in organic content should result in lower (if any) soil binding of Cry proteins.

Estimated Environmental Concentration (EEC): The amounts of Cry1F protein in an acre of corn (if 25,000 corn plants/acre at harvest were left in the field) is approximately 20.5 g/acre. As a result the expected maximum environmental concentration (EEC) of Cry1F protein will be 23 micrograms /kg dry soil (15 cm deep). This does not include any additional Cry protein in the soil as a result of root exudation (if root exudation is shown to occur).

RECOMMENDATIONS: Data for Cry1F protein expression in plant roots and data on Cry protein exudation by roots should be submitted for review.

3) Effects on soil microbial flora

Limited published data do not indicate that Cry proteins have any measurable effect on microbial populations in the soil, even at levels much higher than expected from Cry1F Bt corn cultivation. Due to frequent fluctuations of organic and other inputs into agricultural soil, at any particular time, soil samples are likely to display radically different abundances and diversity of microorganisms. There is no evidence to suggest that the numerous processes mediated by soil microorganisms do not persist across the spectrum from undisturbed soil under native vegetation to intensively cultivated soil under continuous cropping and chemical treatments. Without better information regarding the range of what constitutes natural microbial communities or microbial communities in current agroecosystems, and the consequences of such changes, it is not possible to assign a significance to apparently minor changes in microbial populations when they do occur. Constant fluctuations of soil microbial communities are typical of most soil ecosystems.

Summary: The low concentration of Cry protein in the soil has not been shown to have any adverse effects on non-lepidopteran organisms. Sufficient evidence exists to suggest that adverse impacts of Cry proteins in the soil are not likely, although the levels of expression in the root should be determined to assure that unexpectedly high levels of root expression do not exist. The EEC of Cry1F from corn (23 ：g/kg dry soil) is well below levels used in toxicity tests which were performed at multiples of the expected environmental concentration in the soil.

4) Horizontal Transfer of Trangenes to Plants and Soil Organisms

Microbial transformation with large concentrations of plant transgenes has only been accomplished at low frequencies and under artificial optimized conditions in the laboratory , and only where homology to existing DNA in the recipient bacteria occurs. Under conditions where homology does not occur, horizontal transfer has not been observed. Therefore, DNA transfer occurs rarely if at all from plants to bacteria. In addition, because homologous sequences already exist in soil bacteria (such as native soil *Bacillus thuringiensis*) horizontal transfer of the same sequences from plants, if it were to occur, would not constitute a new phenomenon. Bt species are generally common in soil, if not always abundant, and therefore various *cry* genes have been available for long periods of time for horizontal transfer from Bt to plants or other soil species. Similarly, promoter genes used in making Bt plants have long been present in the soil microorganisms and decaying plant material. Therefore the likelihood of an adverse impact or new horizontal gene transfer that is not already capable of taking place in the soil is extremely unlikely.

b. Risk Characterization for Terrestrial Animals

1) Avian

The dietary LC_{50} value for corn grain (meal) expressing *Bacillus thuringiensis* var. *aizawai* Cry1F protein in corn grain when fed to juvenile northern bobwhite for 5 days was determined to be greater than 100,000 ppm (10% corn meal). The no-observed-effect concentration was also 100,000 ppm. The study is scientifically sound and no treatment mortality or behavior change

was observed between the dosed and control replicates. These data show that there will be no adverse effects on avian wildlife from incidental field exposure to Cry1F corn. These data are, however, not sufficient to make a hazard assessment from repeated exposure(s) to higher doses of Bt corn. The study is rated as supplemental because the concentration tested (10% corn in the diet) is too low to assess hazards to non-target birds from continuous exposure to higher levels of Cry1F protein.

RECOMMENDATIONS: A six week study with 60 to 70% corn in the diet is necessary to assess hazards from chronic exposure of wild and domesticated fowl.

2) Mammalian Wildlife

Since the anticipated exposure of mammalian wildlife is considered high, risk to wild mammals from Bt Cry1F is a potential concern. Direct wild mammal testing, however, is required only when human toxicology data are inadequate for assessment of hazard to wild mammals. The human health effects data submitted to EPA indicate that there is no significant toxicity to rodents from acute oral testing at the maximum hazard dose. In light of this toxicology information, no risk to mammalian wildlife is expected.

3) Plants

Since the active ingredient in this product is an insect toxin (*Bt* endotoxin) that has never shown any toxicity to plants, the plant toxicity studies have been waived.

4) Nontarget Beneficial Organism Studies

a) Honey Bees

The reviewed capped honey bee brood cell study where larvae were fed Cry 1F corn pollen and pure Cry1F protein showed normal larval development and emergence of healthy adult honey bees. This study shows that at levels higher than the expected environmental exposure, the proposed use of Cry1F protein in corn is not likely to have any measurable deleterious effects on the honey bee (*Apis mellifera)*. The data showed no significant difference between treatment mortality or behavior change between the dosed and control replicates. As a result, no discernible detrimental effects to honey bees are expected from the proposed uses of the Cry1F producing corn. The data adequately address potential toxicity concerns for foraging honey bees exposed to Cry1F protein expressed in corn pollen in the field. In addition, since corn is wind pollinated and honey bees do not typically forage field corn, few honey bees are expected to be exposed.

b) Lady beetle predator:

Adult lady beetles (*Hippodamia convergens)* fed a concentration of Bt Cry1F protein at 15x the expected rate found in corn pollen resulted in no mortality or signs of toxicity over a 29 day

period. Therefore, the NOEC was determined to be >15x the concentration of Cry1F found in pollen and the LC_{50} was determined to be > 480 ppm a.i (the test concentration). The submitted study shows that corn containing the Cry1F protein should not cause significant adverse effects to lady bird beetle predators. The test insects were exposed to a dose of active ingredient approximating the amount that would be ingested by the beetles feeding on aphids under field conditions. As a result, no discernible beneficial beetle population effects are expected from the proposed uses of the Cry1F producing corn. This conclusion is confirmed by adult and larval lady beetle abundance found in the field census study. These studies adequately address potential concerns for Cry1F protein expressed in corn to beneficial beetles.

c) Green lacewing

Green lacewing larvae fed a concentration of Bt Cry1F protein at 15x the expected rate found in corn pollen resulted in no mortality or signs of toxicity due to feeding on Cry1F over a 13 day period. Therefore, the NOEC was determined to be >15x the concentration of Cry1F found in pollen and the LC_{50} was determined to be > 480 ppm a.i (the test concentration). These laboratory findings do not show significant detrimental effects and provide data that show a lack of risk to beneficial insects at Cry1F levels that will be encountered in the field use situation. These findings confirm published field studies on the effects of *B.t.* crops on insect predators showing no significant differences in the density of beneficial insects, including green lacewings. The conclusions are also confirmed by the adult and larval green lacewing abundance found in a field census study submitted with this application.

d) Parasitic wasp

Parasitic Hymenoptera *(Brachymeria intermedia*) fed a concentration of Bt Cry1F protein at 10x the expected rate found in corn pollen showed no mortality or signs of toxicity over a 12 day period. Therefore, the NOEC was determined to be >10x the concentration of Cry1F found in pollen. The LC_{50} was determined to be > 320 ppm a.i (the test concentration). As a result, no adverse effect to parasitic wasps are expected from field exposure to Cry1F protein producing corn. The conclusions are also confirmed by the parasitic wasp abundance found in a field census study submitted with this application

e) Monarch butterfly

An additional scientifically sound study submitted by Dow AgroSciences showed that Cry1F is non-toxic to neonate monarch butterfly larvae when fed a #10,000 ng/mL diet dose. First instar larval weight and mortality were recorded after seven days of feeding. There was no mortality to monarchs fed 10,000 ng/mL diet, the highest rate tested. There was some growth inhibition at 10,000 ng/mL diet. Since pollen doses equivalent to 10,000 ng/mL diet are not likely to occur on milkweed leaves in nature, it can be concluded that Cry1F protein will not pose a risk to monarchs.

RECOMMENDATIONS: The conclusions should be confirmed by providing data showing that

the amounts of Cry protein found in pollen on milkweed leaves in the field are at concentrations less than the 10,000 ng/mL diet used in this study. The NOEC of pollen on milkweed leaves also has to be determined.

f) Non-target Insects in the Field

A field study was conducted to determine whether Cry1F Bt corn had any significant negative impact on natural non-target insect populations. Results from a field evaluation study indicate that the transgenic corn lines 1507 and 1360 do not adversely affect the number of beneficial arthropods in the field. In general line 1507 showed larger numbers of beneficial insects. Beneficial insects counted in this study were: lady beetles (*Cycloneda munda* & *Coleomegilla maculata*), predacious Carabids, brown lacewings (Hemerobiidae), green lacewings (*Chrysoperla plorabunda*), minute pirate bugs (*Orius insidiosus*), assassin bugs (Reduviidae), damsel bugs (Nabidae), Ichneumonid and Braconids (parasitic wasps), damselflies and dragonflies, and spiders. Data included counts of adult and larval lady beetles and lacewings. This field census study adequately addresses potential concerns for Cry1F protein expressed in corn to non-target insect populations.

RECOMMENDATIONS: It is recommended that the beneficial insect monitoring should continue into the first few years of commercial use of Cry1F corn crops to confirm the single season "no effects" findings and to gather data on long range non-target insect effects and abundance.

g) Earthworm:

The submitted data show that Cry1F protein has no measurable deleterious effects on earthworms, a representative beneficial soil invertebrate species. This suggests that the proposed uses of the Cry1F protein in corn are not likely to have any measurable population effects on beneficial soil invertebrates. The one limit test concentration of 2.26 mg Cry1F/kg dry soil represented more than 100X the estimated concentration present in the top six inches of an acre of soil following the incorporation of 25,000 senescent corn plants. This concentration is higher than any amount of Cry protein that may be present in the soil during any stage of the growing season (such as from root exudation). Based on the results of this study, Cry1F transgenic corn plantings will have no adverse effects on earthworms.

h) *Collembola*:

Since Collembola feed on decaying plant material in the soil, they may be exposed to Cry1F protein in corn found in the field. A study was conducted to determine if there may be adverse effects of Cry1F on Collembola. The study is scientifically sound and no treatment mortality or behavior change was observed between the dosed and control replicates after 28 days. The results of this study indicate that at levels that would reasonably be expected to be found in the field, collembola were not affected by chronic exposure to Cry1F protein. The exposure rates in this study are 1560-, 388-, and 79-fold-higher than the expected field concentration. The

reviewed data show that *Bacillus thuringiensis* Cry1F corn protein has no measurable deleterious effects on collembola (*Folsomia candida*), a representative beneficial soil insect species. This indicates that the proposed uses of the Cry1F protein in corn are not likely to have any measurable population effects on beneficial soil insects.

c. Risk Characterization for Aquatic Animals

Aquatic species: There is no evidence for sensitivity of aquatic (including endangered) species to Cry proteins. Toxicity studies with *Daphnia magna*, a very sensitive aquatic test organism, show no hazard for fish or invertebrates exposed to either corn pollen or to bacterially expressed Cry1F protein. In addition, aquatic exposure from Bt crops is extremely small. A simple standard pond scenario (1-ha pond, 2-m deep draining a 10-ha watershed planted with corn) was used to develop a worst case EEC for Cry1F protein on the basis of corn pollen loadings from airborne pollen deposition and agricultural runoff. Airborne pollen deposition results in water concentrations of approximately 1.25 ng Cry1F/mL and the contribution of Cry1F to the pond through agricultural runoff is <0.15 ng/mL. Thus, total water concentration of 1.4 ng Cry1F protein/L is projected under worst case conditions

1) Aquatic Invertebrates

The major source of Bt Cry1F protein in fresh water would be corn pollen. Toxicity studies with corn pollen containing Cry1F proteins conducted using the sensitive aquatic indicator species *Daphnia magna* show the no-mortality concentration and NOEC to be >100 mg a.i./L. There were no overt signs of toxicity to daphnids exposed to 100 mg Bt Cry1F pollen/L. The amount of pollen tested was considered to well exceed field exposure. These data indicate that the expected environmental concentration of corn pollen from the proposed use of Cry1F protein in corn is not likely to have any measurable population effects on aquatic invertebrates.

2) Fish

The registrant has requested a waiver of freshwater fish testing for transgenic maize containing *Bacillus thuringiensis* var. *aizawai* (Bt) Cry 1F protein. The basis of the waiver is the lack of significant exposure to fish and the low content of Cry1F protein in corn kernels in commercially manufactured fish diets (in aquafarms). Submitted data show that following processing there were undetectable levels of Cry1F protein in fish food containing Cry 1F maize. The submitted data are sufficient to conclude that the low aquatic EEC and the lack of measurable concentrations of Cry1F protein in commercial fish diets are unlikely to present hazardous exposures to fish. Accordingly the registrant's request to waive fish toxicity studies is acceptable.

3) Estuarine and Marine Animals

The Estuarine fish study was not required for this product because of very low or no potential for

exposure.

d. Impacts on Endangered Species

The primary route of exposure to Cry1F protein in corn is through ingestion of corn tissue. There are no reports of threatened or endangered species feeding on corn plants, therefore such species would not be exposed to corn tissue containing the Cry1F protein. Since Cry1F corn pollen have shown no toxicity at the expected environmental concentration rates (EEC) to mammals, birds, plants, aquatic species, insect and other invertebrate species tested a "may effect" situation for endangered land and aquatic species is not anticipated given the current use pattern for this product. In its evaluation of endangered and threatened species, EPA considered all of the species listed in the Greenpeace and Environment Defense Fund petitions. In addition, EPA does not expect that any threatened or endangered plant species will be affected by outcrossing to wild relatives or by competition with such entities. Hybrid corn does not exist in the wild, nor are there wild plants that can interbreed with corn in the United States.

Because of the selectivity of Cry1F protein for lepidopteran species, endangered species concerns are mainly restricted to the order Lepidoptera. The majority of endangered lepidopteran species have very restricted habitat range that does not encroach on corn production areas. For example,
Mitchell's satyr butterfly occur in wetlands fed by seeps and springs known as fens, and their larvae, which are present throughout the summer, feed primarily on sedges. No Mitchell satyr populations have been seen in close proximity to corn fields.

Examination of an overlay map showing the county level distribution of endangered lepidopteran species (as listed by the U.S. Fish and Wildlife Service) relative to corn production counties in the US, shows that they do not occur in agricultural areas where corn is grown, nor is corn considered a host plant for these species. The overlay map when combined with restricted habitat range clearly indicates that any potential concern for endangered or threatened butterfly species, including those listed in the Greenpeace petition is restricted to the Karner blue butterfly.
The Karner blue is found along the northern extent of the range of wild lupine, where there are prolonged periods of winter snowpack, primarily in parts of Wisconsin, Michigan, Minnesota, Indiana, New Hampshire and New York. The Karner blue requires wild lupine *(Lupinus perennis)* as an oviposition substrate and larval food source, while the adults feed on wild flowers. Wild lupine does not occur in corn fields, although there are anecdotal reports of wild lupine growing 'within a couple of hundred meters of corn fields. Wild lupine grows on dry, sandy soils in pine barrens, oak savannah, forest trails and previously disturbed habitats such as utility rights-of-way, military installations, airports, highway corridors, sand roads and abandoned sand pits. There are recent reports that wild lupine may, in rare instances, grow in the vicinity of corn fields, especially in cases where the field may have been fallow in the previous season. However, there are no reports of Karner blue larvae or wild lupine within one meter of corn fields.

Karner blue oviposition overlap with corn pollen shed is also minimal. Although first generation Karner blues emerge in mid-April, prior to pollen anthesis, second generation larvae emerge in June-July when there may be some overlap with pollen-shed. However, there should be no risk of Karner blue exposure to maize pollen because larvae typically occur on wild lupines in full sunlight in open areas of savannas or barrens and not within corn fields.

Because Cry1F protein is active against Lepidoptera, some activity against the Karner blue at high dose levels would not be surprising. However, data on the levels of Cry1F pollen exceeding the NOEL inside the 1 meter corn field perimeter are not available. Testing of Karner blue larvae directly is difficult due to its endangered status. Although close relatives of the Karner blue butterfly are available, data from related lepidopteran species do not predict susceptibility to low levels of Bt proteins, even within the same genus. Since susceptibility of the Karner blue is not necessarily equivalent to other species from the genus Lycaeides and tests cannot be conducted with the Karner blue, determining a NOEL is difficult to impossible. However, the Karner blue is probably no more sensitive to Cry1F than monarch butterflies and will not consume toxic levels of Bt in the field.

Conclusion: Exposure of Karner blue butterflies to harmful levels of Cry1F corn pollen is not expected. Likewise, a review of the preferred habitats of other lepidopteran species listed as endangered by the U.S. Fish and Wildlife Service, including the endangered Mitchell satyr butterfly, indicates that no exposure to harmful levels of Cry1F protein containing pollen will take place. Therefore, EPA believes that this action will have no effect on listed species. However, because of the lack of direct testing of Cry1F effects on the endangered Karner blue butterfly *(Lyceides melissa samuelis)* and recent information on the possibility of exposure of the Karner blue to corn pollen under certain rare circumstances (such as replanting of fallow fields), at this time geographic restrictions are needed for this product to eliminate potential exposure of Karner blue butterflies to Cry1F corn pollen. Without geographic restrictions, at this time it is not possible to make a definitive "no effect" finding without a consultation with the US Fish and Wildlife Service. The Agency plans to conduct further work to understand the extent to which the practice of replanting fallow fields might expose Karner blue butterflies to Bt corn pollen.

4. Endangered Species Statement

Of particular concern is the endangered Karner blue butterfly (*Lycaeides melissa samuelis*) with populations in Illinois, Indiana, Michigan, Minnesota, New Hampshire, New York, and Wisconsin. Because of the potential for *B. t.* Cry protein containing pollen to affect Lepidoptera adversely, Cry1F maize must not be near habitats of the Karner blue butterfly in the following counties where the Karner blue butterfly is known to exist in scattered populations: Illinois - Lake; Indiana - Porter and Lake; Michigan - Allegan, Lake, Monroe, Montcalm, Muskegon, Newaygo and Oceana; Minnesota - Anoka and Winona; New Hampshire - Merrimack; New York - Albany, Saratoga, Schenectady and Warren; Wisconsin - Adams, Barron, Burnett, Chippewa, Clark, Dunn, Eau Claire, Green Lake, Jackson, Juneau, Kenosha, Marquette, Menominee, Monroe, Oconto, Outagamie, Polk, Portage, Sauk, Shawano, St. Croix, Waupaca,

Waushara, Wood; (this list is from the Wisconsin Statewide Karner Blue Butterfly Habitat Conservation Plan and Environmental Impact Statement). Although it is unlikely that sufficient Cry1F expressing pollen would accumulate on the wild lupine (*Lupinus perennis*) that constitutes the sole food source for the butterfly larvae, this precaution is needed in the lack of adequate data from the field indicating the precise proximity of wild lupine to corn fields in the above named counties.

D. Resistance Management

The following requirements for Cry1F event 1507 are based on the Agency's requirements for Cry1Ab expressing corn. This is due to the possibility of cross-resistance between Cry1Ab and Cry1F. Modifications of these requirements may result following the Agency's comprehensive reassessment of B.t. plant-pesticides.

1) 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:

a) Grower Guides

Grower Guides and/or Product Use Guides must be submitted to the Agency at the time of distribution to growers. 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 2001 (e.g., because the requirements were enacted after printing and distribution of the Grower Guides) must be conveyed via supplemental communications to Cry1F field corn seed customers.

b) Stewardship Agreement (grower agreement).

Each grower who purchases Cry1F field corn seed must be required to sign a Stewardship Agreement, which will obligate the grower to follow the required IRM and non-target insect protection 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).

2) (Stewardship Agreements/Grower 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.

3) For the 2001 growing season, grower agreements (Stewardship Agreements) for Cry1F 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).

4) 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 Cry1F 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 Cry1F 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.

5) The registrants will monitor for the development of resistance using baseline susceptibility data and/or a discriminating concentration assay when such an assay is available. The registrants will proceed with efforts to develop a discriminating concentration assay. The registrants will ensure that monitoring studies are conducted annually to determine the susceptibility of ECB and corn earworm (CEW) populations to the Cry1F 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 Cry1F 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, the registrants will instruct its customers (growers and seed distributors) to contact the registrants (e.g., via a toll-free customer service number) if incidents of unexpected levels of ECB and/or CEW damage occur.

Upon exclusion of the causes specified in section 7a of this document, the registrants will investigate and identify the cause for this damage by local field sampling of plant tissue from corn hybrids that contain Cry1F corn plant-pesticide and sampling of ECB & CEW populations, followed by appropriate in vitro and in planta assays. Upon the registrant's confirmation by immunoassay that the plants contain Cry1F 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 the registrant, the registrant 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 Cry1F 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 Cry1F crystal protein standard for this bioassay will be *Bacillus thuringiensis* subspecies *aizawai*.

2. > 30% survival and > 25% leaf area damaged in a 5-day bioassay using Cry1F-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 the registrant finds it appropriate to alter the criteria specified in the working definition, the registrant must obtain Agency approval in establishing a more suitable definition.

The insect monitoring programs must include Southwestern corn borer (SWCB) and corn earworm (CEW), in addition to European corn borer (ECB). The 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.

6) 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.

7) a) When field resistance has been demonstrated to have occurred, you must stop sale and distribution of Cry1F corn in the counties where the field resistance has been shown until an effective local mitigation plan approved by EPA has been implemented. The registrant assumes responsibility for the implementation of resistance mitigation actions undertaken in response to the occurrence of resistance during the 2001 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 Cry1F corn, that the seed used had the proper percentage of corn expressing Cry1F protein, that the relevant plant tissues are expressing the expected level of Cry1F 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 should extensive field studies and testing to fully scientifically confirm insect resistance be completed before responsive measures are undertaken.

7) b) The registrant will maintain a (confidential) database to track sales (units and location) of its Cry1F corn on a county-by-county basis. The registrant will provide annually, on a CBI basis, sales data for each state indicating the number of units of corn hybrids that contain the registrant's Cry1F corn plant-pesticide that were sold. As part of the overall sales report, the registrant will provide a listing of an estimate of the acreage planted within such states and counties with sales limitations. This information will be provided by January 31 of the year following each growing season.

8) The registrants will provide grower education. The registrants will agree to include an active partnership with such parties as: university extension entomologists and agronomists, consultants, and corn grower groups. The registrants will implement a grower education program (in part, as requested by the registrants, 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 2001 growing season must be provided to the Agency as they become available for distribution. IRM educational materials must be developed and distributed at the same time that growers receive seed. 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 submitted to EPA on April 19, 1999, 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 prohibited from future purchases of Cry1F corn seed.

E. Benefits

Registration of *Bacillus thuringiensis* subspecies Cry1F protein and the genetic material necessary for its production (plasmid insert PHI 8999) in corn is in the public interest because the new pesticide is comparatively less risky to health or the environment than currently registered pesticides and the benefits (including economic benefits) from the use of the new active ingredient exceed those of alternative registered pesticides and other available non-chemical techniques.

V. DATA GAPS

The following data was determined necessary to complete the pending products' database for registration until September 30, 2001.

1) A longer soil degradation study in actual field soil.

There is no evidence to indicate that prolonged exposure to trace amounts of Cry protein in the soil affects non-target organisms. The submitted data do not, however, sufficiently address the issue of residual Cry protein accumulation in the soil. The soil degradation study should be carried out for a longer period of time to determine the duration and the amount of residual Cry 1F protein in agricultural soil. Also, the soil used in the study should be actual field soil containing the microbial flora normally found in the field. This will give a more accurate rate of degradation of the Cry protein in the agricultural environment because microbial populations in the rhizosphere are commonly 100 fold higher than in bulk soil. Bulk soil generally does not support populations of microorganisms as high as those in the rhizosphere or those in soils with high organic content (plant residues). In addition, field soil high in organic content should result in lower (if any) soil binding of Cry proteins.

2) Confirmatory Monarch butterfly data.

An additional scientifically sound study submitted by Dow AgroSciences showed that Cry1F is non-toxic to neonate monarch butterfly larvae when fed a #10,000 ng/mL diet dose. First instar larval weight and mortality were recorded after seven days of feeding. There was no mortality to monarchs fed 10,000 ng/mL diet, the highest rate tested. There was some growth inhibition at 10,000 ng/mL diet. Since pollen doses equivalent to 10,000 ng/mL diet are not likely to occur on milkweed leaves in nature, it can be concluded that Cry1F protein will not pose a risk to monarchs.

The conclusions should be confirmed by providing data showing that the amounts of Cry protein found in pollen on milkweed leaves in the field are at concentrations less than the 10,000 ng/mL diet used in this study. The NOEC of pollen on milkweed leaves also has to be determined.

3) Continuation of beneficial insect field monitoring.

The beneficial insect monitoring should continue into the first few years of commercial use of

Cry1F corn crops to confirm the single season "no effects" findings and to gather data on long range non-target insect effects and abundance.

4) Insect resistance management data.

The registrants will confer with the EPA as the registrants develop various aspects of its resistance management research program. The registrants agree, as a condition of these registrations, to generate data and 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:

a) The registrants must submit available research data on CEW relative to resistance development and the registrants' 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 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 the registrants as the model development and testing is ongoing. The requirement for development of resistance predictive models may be modified if the registrants provide 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 Bta 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.

b) 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.

c) 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.

d) Development of a discriminating concentration (diagnostic concentration) assay for field resistance (field screening) for ECB, CEW and SWCB. Sampling will be done in accordance with the Industry Plan to determine if increases in Cry1F 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.

e) Effects of corn producing the Cry1F delta endotoxin on pests other than ECB, including but

not limited to CEW, fall armyworm, and the stalk borer complex.

f) 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.

g) 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 2001. By January 31, 2002, you must provide the Agency with the results from these investigations.

h) 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:

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

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

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

4) 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?

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

6) 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.

5) Analytical methods and method validation for the Cry1F protein in corn have been received and are acceptable, but additional confirmatory methods and standard post-registration EPA laboratory method validation are required.

Although, Cry1F protein plant root expression and exudation data and a 6 week avian feeding study with 60 -70% Cry1F corn in the diet were identified as deficiencies for a non-expiring full commercial use registration, they are not considered data gaps for a registration expiring on September 30, 2001. At this time in the reassessment process, these data have not been required of other Bt corn plant-pesticide registrants.

VI.. CONTACT PERSON AT EPA

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