REGISTRATION ELIGIBILITY DECISION

Bacillus thuringiensis subspecies *tolworthi* Cry9C Protein and the Genetic Material Necessary for Its Production in Corn (PC Code 006466)

U.S. Environmental Protection Agency
Office of Pesticide Programs
Biopesticides and Pollution Prevention Division

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I. Executive Summary

A. IDENTITY

*Bacillus thuringiensis*subspecies *tolworthi* Cry9C Protein and the Genetic Material Necessary for Its Production in Corn

B. USE/USAGE

The product is for use in field corn against the European corn borer. Planting is limited to 120,000 acres with 11,000 acres of seed production. Use and exposure is limited to industrial (such as ethanol production) and domestic feed only.

C. RISK ASSESSMENT

(1) Human Health Risk Assessment

Based on the toxicology data cited and the limited exposure expected with animal feed use, there is reasonable certainty that no harm will result from aggregate exposure to the U.S. population, including infants and children, to residues of *Bacillus thuringiensis* subspecies *tolworthi* Cry9C protein and the genetic material necessary for its production in corn. This includes all anticipated dietary exposures and all other exposures for which there is reliable information. The Agency has arrived at this conclusion because the tolerance exemption is limited to feed use only. The conclusion of safety is supported by the lack of toxicity after administration of a high oral dose (3,760 mg/kg), the lack of homology to known toxins or allergens, and the minimal to nonexistent exposure via dietary and non-dietary routes.

(2) Ecological Risk Assessment

Studies have been submitted which demonstrate no effects under test conditions to birds (Bobwhite quail), non-target soil organisms (*Collembola* and Earthworm), honey bees, ladybird beetle, aquatic invertebrates (*Daphnia magna*) and beneficial non-target insects in the corn field. In addition, it has been shown that conventional processes used in the commercial preparation of fish food inactivate any Cry9C protein present in corn grain.

Beneficial insect monitoring has been recommended to continue into the first few years of commercial use of the transgenic corn crops to confirm the small plot "no effects" findings. This data is not required as a condition for the limited registration.

(3) Insect Resistance Management

For the limited 120,000 acre registration, the following limitations would be sufficient to mitigate the risk of resistance developing to the Cry9C protein.

1) Refuge requirements reflecting the NC-205 recommendations are required for this product. Most experts in the field, the February 1998 SAP, and the Agency agree that the deployment of refugia must be a requirement of any IRM program. Although there is still uncertainty and disagreement among scientists as to the optimal refuge design for Bt corn, it is recommended that the USDA NC-205 guidelines be mandated for Cry9C corn. These guidelines reflect the current knowledge base among USDA, academic, industry, and EPA scientists. Specifically, a 25% unsprayed or 40% sprayed non-Bt corn structured refuge in close proximity to the Bt crop is recommended. Based upon additional research, the refuge should be established within 1500-2000 feet of the Bt crop.

2) PGS must provide specific information through their technical bulletins, brochures, product labels, and educational presentations so that growers have the necessary tools to successfully implement an IRM plan. A World Wide Web site on the internet would be a practical way to provide specific resistance management information. Included in this IRM information should be instructions on the appropriate use of the Bt plant-pesticides in a resistance management program, compatibility with existing Integrated Pest Management (IPM) programs, refuge deployment and management (including IPM options), monitoring, reporting of unusual pest damage, and any local and regional IRM considerations. The success of any IRM program will ultimately depend on growers who have the knowledge and tools to understand the problem of resistance and the steps that can be taken to combat it.

3) PGS must maintain a (confidential) database to track sales by units and location of Cry9C corn on a state and county-by-county basis. This material should be submitted annually (by January 31 of the year following each growing season) to the Agency on a Confidential Business Information (CBI) basis. As part of this report, PGS should provide an estimate of the acreage for Cry9C corn within each state.

D. DATA GAPS /USE RESTRICTIONS

There are no data gaps for the limited registration.

The registration will automatically expire on midnight May 30, 1999. This will allow sale and distribution of Cry9C corn for the 1998 and 1999 growing season. The registration is for field corn to be used only in animal feed, industrial non-food uses such as ethanol production, and seed increase. In addition, any corn grown within 660 feet of Cry9C corn must also be limited to use in animal feed and industrial non-food uses such as ethanol production.The acreage of corn planted may not exceed 109,000 acres for the animal feed and industrial uses and 11,000 acres for seed increase.

Plant Genetic Systems will assure, via grower agreements, that the USDA NC-205 guidelines for refuge are followed for all Cry9C corn. These guidelines reflect the current knowledge base among USDA, academic, industry, and EPA scientists. Specifically, a 25% unsprayed

or 40% sprayed non-Bt corn structured refuge in close proximity to the Bt crop is required. The refuge will be established within 1500-2000 feet of the Bt crop.

II. Overview

A. ACTIVE INGREDIENT OVERVIEW

Pesticide Name: *Bacillus thuringiensis* subspecies *tolworthi* Cry9C Protein and the Genetic Material Necessary for Its Production in Corn

Trade and Other Names: StarLink

CAS Registry Number: None

OPP Chemical Code: 006466

Registrants: Plant Genetic Systems (America) Inc.
7200 Hickman Road, Ste. 202
Des Moines, Iowa 50322

B. USE PROFILE

Type of Pesticide: Plant-Pesticide

Use Sites: Field Corn

Target Pests: European corn borer

Formulation Types: N/A

Method and Rates of Application: N/A

Use Practice Limitations: Planting is limited to 120,000 acres with 11,000 acres of seed production. Use and exposure is limited to industrial (such as ethanol production) and domestic feed only.

Timing: N/A

C. ESTIMATED USAGE

120,000 acres

D. DATA REQUIREMENTS

The mammalian toxicology and ecological effects data requirements have been fulfilled. Product analysis data requirements are adequately satisfied. The data requirements for granting this registration under Section 3(c)(5) of the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) have been reviewed by the Biopesticides and Pollution Prevention Division (BPPD). Based on submitted information, the Agency foresees no unreasonable adverse effects to human health and the environment from the limited use of this plant-pesticide and recommends an unconditional registration of this new active ingredient for the proposed uses.

E. REGULATORY HISTORY

On 2/5/97, a 3305 acre crop destruct EUP [70218-EUP-R] was granted to Plant Genetic Systems (America) for plantings through 11/30/97. This EUP was extended on 3/5/98 for plantings through 11/30/98 for the same acreage. On 3/26/98, the EUP was amended to allow the use of corn grown under the EUP in animal feed. A temporary exemption from the requirement of a tolerance was promulgated as a final rule under 40 CFR 180.1192 to permit the use of Cry9C corn in animal feed. The rule published 4/10/98 in the Federal Register.

Plant Genetic Systems (America) is a subsidiary of Plant Genetic Systems N.V. of Gent, Belgium. Plant Genetic Systems N.V. was acquired by AgrEvo, although it still retains it's name with the EPA registration application.

F. FOOD CLEARANCES/TOLERANCES

40 CFR 180.1192 is being amended to read as follows:

Sec. 180.1192 *Bacillus thuringiensis* subspecies *tolworthi* Cry9C protein and the genetic material necessary for its production in corn; exemption from the requirement of a tolerance.

The plant-pesticide *Bacillus thuringiensis* subspecies *tolworthi* Cry9C protein and the genetic material necessary for its production in corn is exempted from the requirement of a tolerance for residues, only in corn used for feed; as well as in meat, poultry, milk, or eggs resulting from animals fed such feed.

III. Science Assessment

A. PHYSICAL/CHEMICAL PROPERTIES ASSESSMENT

All product chemistry data requirements for the plant-pesticide are satisfied.

1. Product Identity and Mode of Action

Product Identity: The Agency has classified *Bacillus thuringiensis* Cry9C Protein and the Genetic Material Necessary for Its Production in Corn as a plant-pesticide.

Mode of Action: Insect stomach poison.

2. Food Clearances/Tolerances

40 CFR 180.1192 is being amended to read as follows:

Sec. 180.1192 *Bacillus thuringiensis* subspecies *tolworthi* Cry9C protein and the genetic material necessary for its production in corn; exemption from the requirement of a tolerance.

The plant-pesticide *Bacillus thuringiensis* subspecies *tolworthi* Cry9C protein and the genetic material necessary for its production in corn is exempted from the requirement of a tolerance for residues, only in corn used for feed; as well as in meat, poultry, milk, or eggs resulting from animals fed such feed.

3. Product Characterization

The Cry9C gene was originally isolated from a *Bacillus thuringiensis* subsp. *tolworthi* strain. The gene was then synthesized with plant preferred codons before it was stably inserted into corn plants to produce a truncated and modified Cry9C protein. The tryptic core of the microbially produced Cry9C protein is similar to the Cry9C protein found in event CBH351 save for a single amino acid substitution in the internal sequence and the addition of two amino acids to the N-terminus. The Cry9C protein was produced and purified from a bacterial host to utilize in the mammalian toxicity studies due to the bacterium's greater production potential. Product analysis that compared the Cry9C protein from the two sources included: SDS-PAGE, Western blots, N- terminal amino acid sequencing, glycosylation tests (for possible post- translational modifications) and insect bioassays.

Table #1: Product Characterization

Guideline	Study	*Result*	*MRID #*
N/A	*Composition of Grain from Cry9C Corn Derived from Transformation Event CBH-351, USA 1996*	*Compositional analysis of grain from corn expressing Cry9C and other corn lines sharing similar parental lines indicates the Cry9C corn does not differ from the closely related -Bt corn, the standard hybrid or the reference values published by USDA-HNIS. While there were differences from the reference values for protein, ash and several amino acids, there were no changes among the three groups analyzed that would distinguish the +Bt hybrid as being nutritionally different from the others. CLASSIFICATION: SUPPLEMENTAL. This analysis is not necessary for risk assessment of the pesticidal substance itself. The results may be appropriate for detecting nutritional changes.*	*442581-04*
N/A	Test Substance Characterization	The control plant extracts had protein concentrations from 9.0 mg to 21.0 mg/gram powder or pollen and no detectable levels of either Cry9C or PAT protein (ELISA). The CBH-351 event corn plant powders had protein levels from 13.5 mg to 24.8 mg/gram powder or pollen. The ELISA concentration of Cry9C protein in the powders were 290µg (TPP-351-0396) or 359µg (TPP-351-0196) per gram plant powder. The Cry9C level in pollen was 0.24µg/gram pollen. A European corn borer bioassay of the plant powder gave an LC_{50} value of 31.3 ng/cm² compared to 72.9 ng/ml for the purified bacterial Cry9C protein. The Cry9C protein appears to be stable in lyophilized plant powder for three months (441µg in TPP-351-0196). CLASSIFICATION:ACCEPTABLE. Note: An "interferring substance" in the Cry9C purified bacterial powder was theorized to account for discrepancies between the Bradford, ELISA and OD_{280} protein assays. It is unclear how this interference was resolved to use the bacterial Cry9C as a standard for the ELISA assay or in the spike and recovery experiment (MRID 443844-02).	442581-05
N/A	Determination of Test Substance Equivalence Between Corn Plant Produced Cry9C and Bacterially Produced Cry9C	Cry9C protein from corn, *Bacillus thuringiensis* and *Escherichia coli* was compared for similarity in activity against European corn borer (ECB) and biochemical characters such as molecular weight, immunoreactivity, N-terminal amino acid sequence and post-translational glycosylation. SDS-PAGE indicated a molecular weight of 67.92kD, 70.47kD and 73.79kD for the *E. coli, B. thuringiensis* and corn produced extracts, respectively. Each of the samples has a prominent immunoreactive band at approximately 70kD and other very faint, lower molecular weight bands. The results of the N-terminal amino acid analysis indicate that there is N-terminus protease clipping of from 3 to 7 amino acid residues. The residues actually obtained in the results agree with the expected sequence. Staining for the carbohydrates did not reveal any detectable Cry9C protein glycosylation by the data presented although the company claims there is some minor positive staining. The bioactivity against ECB of Cry9C protein from plant or *B. thuringiensis* in a diet surface contamination assay was similar although the values and stability differed. CLASSIFICATION: ACCEPTABLE.	443844-01

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Guideline	Study	*Result*	*MRID #*
N/A	Validation of the Determination of Cry9C Protein Concentration in Corn Plant Powder	The results of testing the three types of extracts indicate the ELISA assay displayed a response over the range of 50 to 250µg/ml Cry9C protein that could be plotted as linear (r^2=>0.95) but appears in most cases to be a typical shallow sigmoid curve forced through the origin. The assay showed a similar response function regardless of the extract tested. The percent recovery for two quality control samples of 78.6 and 183.4 µg/ml ranged from 61.5% and 87.5% in the extraction buffer alone to 59% and 73% in plant protein matrix to 58% and 79% in the total plant powder, respectively. These results indicate a minor interference with the plant protein matrices and a potential recovery of 60% to nearly 80% of the Cry9C protein present. CLASSIFICATION:ACCEPTABLE. We note that these results were not used to correct Cry9C protein values given in other assays. See the Ecological Exposure and Risk Characterization section.	443844-02
N/A	Protein Chemistry: Molecular Characterization of the Cry9C Corn Transformation Event CBH-351	Southern analysis of the DNA from event CBH-351 corn indicates that both the *cry9c* and *bar* genes are incorporated into the genome. The *cry9c* gene is found as a single copy whereas the *bar* gene is found in multiple copies (apparently four). Northern analysis of the mRNA indicate that *cry9c* is expressed in leaf, stem, root, tassel, and ear. Concentrations of the mRNA present indicate there is a variability in the tissues sampled. There is no indication about the possible variability of Cry9C expression with different developmental stages in the plant. It is unclear if the mRNA levels correlate to Cry9C protein expression levels. Northern analysis indicate that no mRNA from the *bla* gene is detectable as would be expected from a gene under control of a bacterial promoter. CLASSIFICATION: ACCEPTABLE.	442581-01
N/A	*In Vitro* Digestibility and Heat Stability of the Endotoxin Cry9C of *Bacillus thuringiensis*	The samples of lyophilized Cry9C protein expressed in corn showed no signs of protein disintegration when subjected to *in vitro* digestion in simulated mammalian gastric fluid. These digestions were done either with of without pepsin in the low pH buffer and were assayed by western blot from samples taken at several time points from the mixing the reagents to after 4 hours exposure to the digestive fluids. The same 70kD double band seen in the original Cry9C protein in plant tissue at time 0 was also seen, undiminished , in all the subsequent incubation samples. No effect on Cry9C activity as determined by bioassay was seen after any heat treatment. The most stringent heat treatment was 90°C for 10 minutes. CLASSIFICATION: ACCEPTABLE.	442581-08
N/A	The *BLA* Gene and β-lactamase Gene Product: An Overview	This submission provides a useful review of the mechanisms and importance of antibiotic resistance. This has not been considered a relevant topic for risk assessment of plant-pesticides due to the lack of an expressed β-lactamase protein in the transformed plant (the gene is controlled by a bacterial promoter) and the extremely low probability of any transfer of the resistance factor gene to the resident gut bacteria. CLASSIFICATION: SUPPLEMENTAL. This submission provides literature information about antibiotic resistance not necessary for risk assessment of this plant-pesticide. No more data need be submitted for this subject.	442581-02

Guideline	Study	*Result*	*MRID #*
N/A	Amino Acid Sequence Homology Search with the Corn Expressed Truncated Cry9C Protein Sequence	Three hundred sequences were listed as having regions of homology with the 626 amino acids of the Cry9C truncated toxin protein. The first 64 proteins in the list were all parasporal proteins from *Bacillus thuringiensis* otherwise known as δ-endotoxins. Other δ-endotoxins were found at 67, 76, 78, 79, and 80. These proteins had regions of homology that gave a "significant homology". The table of values indicated a matching score above 4 standard deviations would contain all the δ-endotoxins mentioned above. The algorithim for converting the matches and penalties into homology scores was not described although it was stated that " all other proteins (besides the δ-endotoxins referred to above) have less than 20% exact sequence matching and no major stretches of sequence homology could be detected, indicating that in these cases the sequence homology is not significant." CLASSIFICATION: SUPPLEMENTAL. BPPD has not yet finalized requirements regarding amino acid sequence homology for risk assessment purposes. No further studies need to be done at this time for analyzing homology.	442581-09
N/A	Expanded Molecular Characterization of the Cry9C Corn Transformation Event CBH-351	Tissue samples included in these new Northern blots include mRNA from wild-type, non-transformed corn (B73), root tissue in two CBH351 plants and dry seeds (lot 96ZM001879). The cry9C and bar Northern blots both suggest that mRNA for those traits can be found in leaf, root and stem tissue but is not detectable in the dry seeds or control non-transformed plants. These blots indicate no mRNA from the traits found in CBH-351 plants detectable in the B73 control samples. The blots also indicate that plant to plant variation in mRNA levels may not be as great as suggested by the first submission (MRID 442581-01) where the number of plants actually sampled was not mentioned. That no detectable mRNA can be found in dry seeds is expected when examining a metabolically quiescent tissue like mature seed. Without an indication how mRNA levels relate to protein expression levels, these results do not significantly alter the evaluation from the previous review. Additional information relates to the stability of the introduced traits by Southern blot analysis. The Southern blots show essentially identical banding patterns from the HindIII digests of genomic DNA from CBH-351 corn probed with pRVA9906. None of the designated B73 wild type corn showed any reactivity with the pRVA9906 probe indicating an absence of any traits from that plasmid. The DNA samples analyzed include a series stated as being derived from different genetic backgrounds. Another set of DNA samples was derived from five generations of traditional crosses. The nomenclature used to designate the plant progeny samples was not apparent from the figure legends. CLASSIFICATION:ACCEPTABLE	443844-03

N/A	Cry9C Bacillus thuringiensis Insecticidal Protein Identification of Sequence Homology with Allergenicity by Searching Protein Databanks	Sequence identity for any of the eight amino acid regions in Cry9C was found only to other Bt crystal proteins. No match between any 8 amino acid sequence in Cry9C and any of the allergenic proteins known in the SWISS protein database was found. This lack of homology at a finer level of examination is further evidence that Cry9C is not related to known allergens using a structural consensus approach. CLASSIFICATION: SUPPLEMENTAL. BPPD has not yet finalized requirements regarding amino acid homology for risk assessment purposes. No further studies need to be done at this time for analyzing homology.	443844-04

B. HUMAN HEALTH ASSESSMENT

There is a reasonable certainty that no harm will result from exposure to *Bacillus thuringiensis* subspecies *tolworthi* and the genetic material necessary for its production in corn . This includes all anticipated dietary exposures and all other exposures for which there is reliable information.

1. Toxicology Assessment

a. Acute Toxicity

Table # 2: Toxicological Endpoints

Guideline No.	Study	Result	MRID #
81-1	An Acute Oral Toxicity in Mice with Cry9C Protein as Purified from *Bacillus thuringiensis* Cry9C.PGS2	There were no deaths in any test animals due to test material given at the dose of 3,760 mg/kg during the 14 day observation period. One male mouse displayed hair loss between days 2 and 5. One female displayed decreased activity on the day of dosing. Another female displayed decreased activity , wobbly gait, decreased feces and felt cool to the touch. A third female displayed decreased feces on day 1. All the male mice gained weight during the test period (except during the pre-dosing fast period). Two female mice failed to gain weight between day 0 (prefasted weight) and day 7 and three failed to gain weight between day 7 and day 14. One female did not recover her pre-fasting body weight by day 14. CLASSIFICATION: Acceptable.	442581-07

b. Mutagenicity and Developmental Toxicity

The acute oral toxicity data submitted support the prediction that the Cry9C 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

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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 Cry9C protein is not considered toxic.

c. Subchronic Toxicity

The acute oral toxicity data submitted support the prediction that the Cry9C 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 Cry9C protein is not considered toxic.

d. Chronic Exposure and Oncogenicity Assessment

The acute oral toxicity data submitted support the prediction that the Cry9C 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," RegulatoryToxicology 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 Cry9C protein is not considered toxic.

e. Effects on the Immune and Endocrine Systems

EPA does not have any information regarding endocrine effects for these kinds of pesticides at this time. The Agency is not requiring information on the endocrine effects of these plant-pesticides at this time; and Congress allowed 3 years after August 3, 1996, for the Agency to implement a screening and testing program with respect to endocrine effects.

Regarding toxicity to the immune system, the acute oral toxicity data submitted support the prediction that the Cry9C 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 Cry9C protein is not considered toxic.

Since Cry9C 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 (have a carbohydrate group attached) and present at high concentrations in the food. Data has been submitted which demonstrates that the Cry9C protein is present in low concentrations in corn. However, it is resistant to degradation by heat, acid, and proteases. Additionally, although staining for the carbohydrates did not reveal any detectable Cry9C protein glycosylation by the data presented, the company claims there is some minor positive staining for the presence of carbohydrates. The best available information on the uptake of intact proteins from the diet would indicate that the intact Cry9C protein would not be available in products from animals fed corn products containing Cry9C protein. In addition, homology searches indicated a lack of homology to known toxins or allergens.

Table # 3: Allergenicity

Guideline No.	Study	Result	MRID #
N/A	*In Vitro* Digestibility and Heat Stability of the Endotoxin Cry9C of *Bacillus thuringiensis*	The samples of lyophilized Cry9C protein expressed in corn showed no signs of protein disintegration when subjected to *in vitro* digestion in simulated mammalian gastric fluid. These digestions were done either with of without pepsin in the low pH buffer and were assayed by western blot from samples taken at several time points from the mixing the reagents to after 4 hours exposure to the digestive fluids. The same 70kD double band seen in the original Cry9C protein in plant tissue at time 0 was also seen, undiminished , in all the subsequent incubation samples. No effect on Cry9C activity as determined by bioassay was seen after any heat treatment. The most stringent heat treatment was 90°C for 10 minutes. CLASSIFICATION: ACCEPTABLE.	442581-08
N/A	Cry9C Bacillus thuringiensis Insecticidal Protein Identification of Sequence Homology with Allergenicity by Searching Protein Databanks	Sequence identity for any of the eight amino acid regions in Cry9C was found only to other Bt crystal proteins. No match between any 8 amino acid sequence in Cry9C and any of the allergenic proteins known in the SWISS protein database was found. This lack of homology at a finer level of examination is further evidence that Cry9C is not related to known allergens using a structural consensus approach. CLASSIFICATION: SUPPLEMENTAL. BPPD has not yet finalized requirements regarding amino acid homology for risk assessment purposes. No further studies need to be done at this time for analyzing homology.	443844-04

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Guideline No.	Study	Result	MRID #
N/A	Investigation of Allergens in Wild-Type and Transgenic Corn	The 21 sera samples from suspected corn-sensitive individuals all tested positive in the RAST assay by having >3% reactivity. The transgenic and wild-type aqueous corn extracts were not obviously different in responsiveness for individuals and a t-test of the RAST % reactivity did not reveal any significant differences. The RAST inhibition assay gave results indicating that both wild type and transgenic corn extracts gave substantial inhibition of the wild type corn RAST. Statistical analysis of the inhibition curves generated for RAST inhibition from wild type versus transgenic corn extracts did not indicate significantly different 50% inhibition values, slopes or y-intercepts. The type of extract, aqueous or alcoholic, utilized in the inhibition assays was never specified. Both the wild type and transgenic aqueous corn extracts gave higher levels of reactivity in the immunoblot assay than the alcoholic extracts. A comparison of the IgE reactions for specific corn atopic individuals indicated that there were similar reactive banding patterns in both transgenic and wild type corn. In some individuals there were a greater number of reactive bands ranging in molecular weight whereas in others there were only one or two bands, generally of lower molecular weight, which had very significant staining. There was no identification of individuals in the SDS-PAGE lanes so no correlation between the intensity of the % reactivity in RAST and the number or intensity of staining in the immunoblot assay could be made. A two-fold dilution series with a pool of 10 RAST positive corn atopic sera was tested against the wild type and transgenic corn extracts. The pattern of reactivity was very similar between the transgenic and wild type extracts with the intensity of the reaction again being higher for the aqueous versus alcoholic extracts. There were some unique bands present in either the wild type or transgenic extracts but since these bands did not show detectable effects on the serum reactivity kinetics in the RAST or RAST inhibition assays it is difficult to judge the importance of their presence. CLASSIFICATION:SUPPLEMENTAL. This study does not address the potential for inducing food allergy from a novel protein lacking a history of dietary exposure. An additional control testing purified Cry9C protein against corn atopic sera should have been included to establish the negative reactivity background. The study does establish a baseline of corn allergen reactivity for subsequent comparisons if such an allergic response does occur over time.	443844-05

Guideline No.	Study	Result	MRID #
N/A	Amino Acid Sequence Homology Search with the Corn Expressed Truncated Cry9C Protein Sequence	Three hundred sequences were listed as having regions of homology with the 626 amino acids of the Cry9C truncated toxin protein. The first 64 proteins in the list were all parasporal proteins from *Bacillus thuringiensis* otherwise known as δ-endotoxins. Other δ-endotoxins were found at 67, 76, 78, 79, and 80. These proteins had regions of homology that gave a "significant homology". The table of values indicated a matching score above 4 standard deviations would contain all the δ-endotoxins mentioned above. The algorithim for converting the matches and penalties into homology scores was not described although it was stated that " all other proteins (besides the δ-endotoxins referred to above) have less than 20% exact sequence matching and no major stretches of sequence homology could be detected, indicating that in these cases the sequence homology is not significant." CLASSIFICATION: SUPPLEMENTAL. BPPD has not yet finalized requirements regarding amino acid sequence homology for risk assessment purposes. No further studies need to be done at this time for analyzing homology.	442581-09

2. Dose Response Assessment

No toxicological endpoints are identified.

3. Dietary Exposure and Risk Characterization

Based on the toxicology data cited and the limited exposure expected with animal feed use, there is reasonable certainty that no harm will result from aggregate exposure to the U.S. population, including infants and children, to residues of *Bacillus thuringiensis* subspecies *tolworthi* Cry9C protein and the genetic material necessary for its production in corn. This includes all anticipated dietary exposures and all other exposures for which there is reliable information. The Agency has arrived at this conclusion because the tolerance exemption is limited to feed use only. The conclusion of safety is supported by the lack of toxicity after administration of a high oral dose (3,760 mg/kg), the lack of homology to known toxins or allergens, and the minimal to nonexistent exposure via dietary and non-dietary routes.

(a) Aggregate Exposure

The available information on the aggregate exposure levels of consumers (and major identifiable subgroups of consumers) to the Cry9C protein residue include dietary exposure and exposure from non-occupational sources. Exposure via the skin or inhalation is not likely since the Cry9C plant-pesticide is contained within plant cells essentially eliminating these exposure routes or reducing these exposure routes to negligible. Drinking water is unlikely to be significantly

contaminated with Cry9C protein due to the low expression of the protein in corn tissue, degradation of plant materials in the soil and low leaching potential of a protein from a soil matrix. Minimal to non-existent oral exposure could occur from ingestion of meat, poultry, eggs or milk from animals fed corn containing the plant-pesticide and from drinking water. While unlikely, meat, eggs or milk from animals fed corn containing the plant-pesticide could contain negligible but finite residues. This is viewed as a remote possibility due to the low Cry9C expression level in corn tissue (3 to 250 µg/gm dry weight), the anticipated degradation and elimination of the Cry9C protein by the animal or the lack of uptake of such a large protein by the animal's intestinal tract. It is not possible to establish with certainty whether finite residues will be incurred, but there is no reasonable expectation of finite residues. However, the best available information on the uptake of intact proteins from the diet would indicate that the intact Cry9C protein would not be available in products from animals fed corn products containing Cry9C protein.

(b) Cumulative Effects

The Agency 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 adults as well as on infants and children of such residues and other substances with a common mechanism of toxicity. Since there is no indication of mammalian toxicity to the Cry9C protein from the studies submitted, there is no reason to believe there would be cumulative toxic effects.

(c) Safety Determination

The tolerance exemption is limited to residues of the Cry9C protein resulting from feed use only. The basis of safety for this tolerance exemption includes both the results of the acute oral study at high doses indicating no toxicity and the anticipated minimal to nonexistent human dietary exposure of the Cry9C protein via animal feed use. Bt microbial pesticides, containing Cry proteins other than Cry9C, have been applied for more than 30 years to food and feed crops consumed by the U.S. population. There have been no human safety problems attributed to the specific Cry proteins. An oral dose of the tryptic core Cry9C protein of at least 3,760 mg/kg was administered to 10 animals without mortality, thus demonstrating a high degree of safety for the protein. Transient weight loss in three female rodents was observed, but not in any males. Transient weight loss has been observed in similar studies conducted on other purified Cry proteins as well as microbial pesticides and this is not considered a significant adverse effect.

A comparison of the amino acid sequence of the Cry9C protein with those found in the PIR, Swiss-Prot and HIV AA data bases did not reveal any significant homology with known toxins or allergens. The in vitro digestibility study showed the Cry9C protein to be stable to pepsin at pH 2.0. The Cry9C protein was shown to be stable to heat at 90 degrees C for 10 minutes. The Cry9C protein in corn is the trypsin resistant core and should therefore be stable to tryptic digest, however no data

was submitted to demonstrate this. The best available information to date would indicate that edible products derived from animals such as meat, milk and eggs, intended for human consumption, have not been shown to be altered in their allergenicity due to changes in the feed stock utilized. This information would include no transfer of allergenic factors from cattle fed soybeans or corn to the derived meat or milk eaten by individuals with food sensitivity to soybeans or corn.

(d) Infants and Children

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 provides that EPA shall apply an additional tenfold margin of exposure (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 exposure (safety) will be safe for infants and children. In this instance, based on all the available information, the Agency concludes that infants and children will consume only minimal, if any, residues of this plant-pesticide and that there is a finding of no toxicity. 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.

4. Occupational Exposure and Risk Characterization

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. If negligible exposure should occur, the Agency concludes that such exposure would present no risk due to the lack of toxicity

C. ENVIRONMENTAL ASSESSMENT

1. Ecological Effects Hazard Assessment

Studies have been submitted which demonstrate no effects under test conditions to birds (Bobwhite quail), non-target soil organisms (*Collembola* and Earthworm), honey bees, ladybird beetle, aquatic invertebrates (*Daphnia magna*) and beneficial non-target insects in the corn field. In

addition, it has been shown that conventional processes used in the commercial preparation of fish food inactivate any Cry9C protein present in corn grain.

Beneficial insect monitoring has been recommended to continue into the first few years of commercial use of the transgenic corn crops to confirm the small plot "no effects" findings.

Table # 4: Non-Target Toxicity Studies

Guideline No.	Study	Result (LC_{50})	Status, Classification & Comments	MRID #
OPPTS Series 885-4380	Cry9C protein in corn pollen: A dietary toxicity study with the honey bee (*Apis mellifera*)	LC_{50} >5.8 µg Cry9C protein/L diet	This study is acceptable, and determined that the dietary LC_{50} for honeybees exposed to Cry9C protein in corn pollen for 8 days was greater than 5.8 µg Cry9C protein/L diet [24,000 µg corn pollen/mL]. The no-observed-effect concentration was 5.8 µg Cry9C protein/L diet.	443843-02
71-2, 154-7	Corn Plant Powder Containing Cry9C Protein: A Dietary Toxicity Study with the Northern Bobwhite	LC_{50} > 58 µg Cry9C protein/g diet	This study was conducted according to accepted protocols and determined that the dietary LC_{50} for northern bobwhite exposed to corn plant powder containing Cry9C protein was greater than 58 µg Cry9C protein/g diet, when administered in a diet containing 20% (w/w) of the powder, the only concentration tested. The no-observed-effect and no-mortality concentrations were 58 µg Cry9C protein/g diet.	442581-14
72-2, 154-9	Freshwater Aquatic Invertebrate Acute Bioassay	Actual LC50 could not be determined.	The study was conducted according to accepted protocols and determined that the 48-hour EC_{50} of Cry9C protein in corn pollen is >0.036 µg Cry9C protein/L (150 mg Bt plus corn pollen/L). The NOEC and the no mortality/immobility concentration are 0.036 µg Cry9C protein/L (150 mg Bt plus corn pollen/L). Since the test and control solutions appeared cloudy with yellow particles in suspension and settled on the bottom of the test chambers, the study is graded supplemental since the amount of pollen that the Daphnia were exposed to could not be determined. However, the amount Daphnia were exposed to is considered far greater than the EEC and no adverse affects were noted.	442581-12
N/A	Preparation and characterization of catfish pellets.	N/A	Based on results of a protein-specific ELISA analysis, no Cry9C protein was detectable in catfish pellets processed from corn kernels containing Cry9C protein.	443843-01

N/A	Chronic Exposure of *Folsomia candida* to Corn Tissue or Bacteria Expressing Cry9C Protein.	N/A	This study was conducted according to an acceptable protocol and determined that the LD_{50} of *corn plant* Cry9C protein and *bacterial* Cry9C protein to collembola (*Folsomia candida*) over a 28-day exposure period is greater than 50% (by weight) of the diet [brewers yeast and test material], the highest [] tested. The no-effect-level for *corn plant* Cry9C protein was 50% of the diet (180 mg/kg dry soil) . The no-effect-level for *bacterial* Cry9C protein was 5% of the diet (20 gm/kg dry soil). Little mortality was observed in the two Cry9C-amended treatments. There was less than 15% mortality in either of the two 50% Cry9C treatments. There was no statistical difference in mortality rates between any treatment with the test materials and the control treatment. With the exception of the thiodicarb treatment, large numbers of collembola were produced. There were no significant differences in the number of collembola produced between test material treatments and the control for the two lower rates (0.5 and 5%). There was a significantly higher number of collembola recovered from the control treatment than from either of the corn plant Cry9C, the bacterial Cry9C or control plant treatments at the highest rate (50%). Recoveries from each of the 50% Cry9C treatments were statistically similar. The only statistically significant collembola increase rate reduction was from the 50% bacterial Cry9C treatment. Even at the very high rates of exposure used in this test (up to 5 orders of magnitude higher than the EEC), collembola were not acutely affected by chronic exposure to Cry9C protein. Notation is being made that the study results stated that mortality in the yeast control replicates ranged up to 15.4%. Data in Appendix D (MRID 44258110, p. 65) indicate that mortality in the yeast-only controls ranged from 0.0 to 16.7%. This issue is not expected to have any effect on the conclusions reached from this study.	442581-10
N/A	Cry9C Protein in Plant Powder: An Acute Toxicity Study with the Earthworm in an Artificial Soil Substrate.		This study was conducted according to OECD Guideline 207 and determined that the 14-day LC_{50} value for earthworms exposed to corn plant powder containing Cry9C protein in an artificial soil substrate was greater than 1.84 mg Cry9C protein/kg dry soil, the only concentration tested. The no-observed-effect concentration was determined to be 1.84 mg Cry9C protein/kg dry soil.	442581-13

OPPTS Series 885.4340	Cry9C Protein in Corn Pollen: A Dietary Toxicity Study with the Ladybird Beetle (*Hippodamia convergens*).	LC_{50} > 0.36 µg Cry9C protein/L diet (1500 µg corn pollen/mL)	The dietary LC_{50} for Cry9C protein in corn pollen when fed to ladybird beetles for 21 days was determined to be greater than 0.36 µg Cry9C protein/L diet (1500 µg corn pollen/mL), the only concentration tested. The no-observed-effect concentration was 0.36 µg Cry9C protein/L diet.	442581-11
N/A	Insect Host Range Comparison of Cry9C Protein.		This non-guideline study is classified supplementary; it was conducted following GLP regulations 40 CFR 160. The study compared endotoxin activity of Cry9C from three sources (bacteria, corn plant tissue, and corn pollen) by determining the relative sensitivities of larvae of four species of insects. The rank order of sensitivity to bacterial and corn plant tissue for three out of four species was the same; however, the magnitude of the activity differed somewhat. The study also showed that, for two out of three species, the toxicities of Cry9C protein from two different sources, bacteria and corn plant tissue, were similar. Cry9C protein from pollen was at a concentration that did not produce significant toxicity. Toxicity to Corn Plant Cry9C and Bacterial Cry9C in ng Cry9C/cm^2 of diet European corn borer 35.0, 24.2 Tobacco budworm 403, 47.7 Diamondback moth 17.3, 70.2 Corn earworm >200, >1350 (highest doses tested)	442581-06
N/A	Effects of Cry9C Corn on Predatory Non-Target Beneficial Insects and Endangered Species; Determination of Predatory Non-Target Beneficial Insect Study/Pollen Production Study.		This study is classified as supplementary. It was not conducted in accordance with FIFRA Good Laboratory Practices Standards (GLPs) (40 CFR Part 160, 17 August 1989); however, it was conducted to meet the spirit of the GLPs. Deviations from protocol did not affect the quality or the results of the study. There were no significant differences in the numbers of predatory insects trapped or observed on the sprayed versus the unsprayed corn plots (used a non-specific insecticide - Pounce™). There was no consistent pattern of differences in abundance of predatory insects on the Bt+ versus the Bt- corn plots. The most common predator observed was *Coleomegilla maculata*. Trapping height had no significant impact on the number of predators captured. However, testing of larger plot sizes would produce more significant results. RECOMMENDATIONS: Larger plot sizes should be tested to confirm the responses of predatory insects to Bt+ corn; in particular, to see if the lower abundance of predatory insects on the sticky traps in the Bt+ plot is biologically significant.	442581-15

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2. Outcrossing and Weediness

a. Potential for Outcrossing and Weediness

Although corn is thought to have descended from a wild weedy species, corn today cannot exist in the wild as a weed because the female inflorescence, or the ear, restricts seed dispersal. Corn is an open pollinating (cross-fertilizing) species, which probably descended from teosinte, which is more weedy, has more tillers, and does not have ears, as such.

b. Potential for Outcrossing with Wild Zea Species

Teosinte Like corn, teosinte also has 10 chromosomes, is wind (open) pollinated, and tends to outcross, but is a highly variable species genetically compatible and interfertile with corn. Corn and compatible species of teosinte freely hybridize when in proximity to each other. In Mexico and Guatemala, teosinte exists as a weed around the margins of corn fields. A frequency of one F1 hybrid (corn x teosinte) for every 500 corn plants, or 2-5% of the teosinte population, has been reported. The F1 hybrid is robust, fertile, and capable of backcrossing to corn. However, except for special plantings, teosinte is not present in the U.S. Its natural distribution is limited to Mexico, and Guatemala.

Tripsacum *Tripsacum*/corn hybrids have not been observed in the field, but have been accomplished in the laboratory using special techniques under highly controlled conditions. The risk of *Tripsacum*/corn hybrids in the field is considered minimal. Tripsacum/teosinte hybrids have not been able to be produced. Tripsacum species are perennials and seem more closely related to the genus *Manisurus* than either corn or teosinte. *Tripsacum*/corn offspring, when they occur, display various levels of sterility. Of the 16 species of *Tripsacum* described, one is native to the southern tip of Florida, 12 are native to Mexico and Guatemala, and 3 are native to South America.

c. Potential for Outcrossing with Cultivated Zea Varieties

Corn pollen has been shown to travel up to 2 miles under favorable wind conditions. All corns will interpollinate except for certain popcorn varieties. Corn pollen germinates almost immediately after pollination and completes fertilization within 24 hours. Thus corn pollen is highly promiscuous and certification standards for distances between different corn genotypes have been established to maintain desired levels of purity in the production of hybrid corn (a minimum of 660 feet). Commercial plantings of the corn expressing the *Bt* plant-pesticide would result in expression of the Cry9C gene in the seed of other corn plants. Although expression of the Cry9C δ-endotoxin would confer a degree of insect resistance to the

transformed plants, there are other traits which preclude any significant risk of *Bt* maize plants becoming weeds as a result of the expression of the toxin. Cultivated maize has been bred for survival under cultivation only, and is limited in its ability to proliferate as a weed or to survive in the wild by such traits as a non-shattering seed habit, lack of seed dormancy, and lack of cold hardiness.

d. Weediness of Corn

Transformation causes no change in a corn plant's inability to exist as a weed. Likewise, the ability to outcross with teosinte and tripsacum (under carefully controlled conditions) will not be changed. 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. However, no such case has been known or reported in the U.S.

Gene exchange between cultivated corn and transformed corn would be similar to what naturally occurs at the present time within cultivated corn. 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.

3. Ecological Exposure and Risk Characterization

The values reported for environmental expression and fate (as well as plant tissue test material Cry9C concentration) do not take into account the 60-80% recovery estimates from the plant-tissue spike and recovery study [Validation of the Determination of Cry9C Protein Concentration in Corn Plant Powder, MRID No. 443844-02]. This was not deemed significant due to the sensitivity of the method and the fact that the Cry9C protein concentration in the test material and in the estimated environmental concentrations were all unadjusted. All figures may under estimate the exact Cry9C concentration.

a. Ecological Exposure

1) Maximum Expression of Cry9C Protein in Various Corn Tissues

Transgene expression was found throughout the different plant tissues across the growing season. The level of the Cry9C and PAT proteins is stable in tissues and in whole plants during the first two stages - vegetative growth through pollen shed and declined with each of the two final stages, silage and harvest. The decline is less for the Cry9C protein than the PAT protein. Similar trends were observed for the tissue samples. The amounts of Cry9C protein in whole plant for each stage were: vegetative - 250 (μg Cry9C protein /g plant tissue on a dry weight basis) , pollen shed - 230 (μg/g), silage - 96 (μg/g), harvest - 22 (μg/g). The amounts of Cry9C protein in an acre of corn

containing 25,000 corn plants/acre during the vegetative, pollen shed, silage, and harvest stages were 103g , 334g, 495g, and 99g (tissue dry weight basis), respectively.

Based on data submitted, it appears that the expression of the Cry9C δ-endotoxin occurs at the highest levels in leaves, tassel, and whole plants. The maximum levels detected in any individual samples were 175.0 μg/g dry weight for tassel, 44.0 μg/g for leaves, 25.87 μg/g for root, 18.6 μg/g for kernel, 2.8 μg/g for stalk, 0.24 μg/g for pollen and 250.0 μg/g for whole plants.

2) Estimated Half-Life and EEC

The submitted study establishes that the *Bt* protein was active in the test soil mix. Based on a bioassay with the tobacco budworm (*Heliothis virescens*), a target species, whole corn plant Cry9C proteins incorporated into test soils biodegraded over a 42-day period with a half-life of approximately 4.5 days (range, 0.49-12.65 days). This half-life is very comparable with the 4-7 days reported by Palm (Palm, C. J., K. Donegan, D. Harris, and R. J. Seidler. 1994. Quantification in soil of *Bacillus thuringiensis* var. *kurstaki* δ-endotoxin from transgenic plants. Molecular Ecology 3:145-151) for CryIA(c) protein in plant tissue in soil.

Given that the amount of Cry9C protein at harvest is 99 g/ac, the expected environmental concentration (EEC) of Cry9C will be 0.11 mg/kg dry soil (15 cm deep).

Table# 5: Environmental Fate Studies

N/A	Characterization of Cry9C and PAT protein levels in CBH-351 Bt corn under field conditions.		The study characterized the insecticidal Cry9C protein and a marker protein (PAT: phosphinothricin acetyltransferase, coded by a bar gene) in transgenic corn grown under actual field conditions in Johnston, Iowa. Corn plants which express Cry9C and PAT proteins have descended from transformation event CBH-351. The levels of all proteins were fairly stable during the first two sampling stages, vegetative and pollen shed, and declined with each of the two final stages, silage and harvest. This decline over time was true for four other tested strains although data for only two of the stages, vegetative and silage, were provided. The amounts of Cry9C protein in an acre of corn containing 25,000 corn plants/acre during the vegetative, pollen shed, silage, and harvest stages were 103, 334, 495, and 99 g (tissue dry weight basis), respectively. ADEQUACY OF STUDY: Acceptable (Supplementary)	442581-03

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N/A	Environmental fate of Cry9C protein incorporated into soil.		Based on a bioassay with the tobacco budworm (*Heliothis virescens*), a target species, Cry9C protein incorporated into Drummer Ap loam soil [as corn plant tissue containing the Cry9C gene incorporated from *Bacillus thuringiensis* subsp. *tolworthi* (0.368 μg Cry9C/mg tissue)] biodegraded over a 42-day period with a half-life of approximately 4.5 days (range, 0.49-12.65 days). ADEQUACY OF STUDY: Acceptable (Core)	441617-01

b. Risk Characterization for Terrestrial Animals

1) Avian

The consumption of *Bacillus thuringiensis* subsp. *tolworthi* Cry9C protein containing corn has no measurable deleterious effects on bobwhite quail, a representative avian species. This suggests that the proposed uses of the Cry9C protein in corn is not likely to have any measurable population effects on avian wildlife.

An dietary LC_{50} > 58 μg /g diet indicates that Cry9C protein is practically nontoxic to northern bobwhite hatchlings. No treatment mortality, differences in food consumption or behavior was observed between the dosed and control birds. The study, together with the Agency's previous experience of no avian toxicity from *B.t.* Cry proteins incorporated into plants, adequately address potential avian toxicity concerns for Cry9C protein expressed in corn and BPPD believes that no additional avian studies should be needed in order to complete the avian risk assessment.

2)Mammalian Wildlife

These studies are required only when human toxicology data are inadequate for assessment of hazard to wild mammals. The data submitted to EPA indicate that there is no significant toxicity to rodents from acute oral testing at the maximum hazard dose. Since the anticipated exposure of mammalian wildlife is considered high, risk to wild mammals from the *Bt* Cry9C endotoxin is a potential concern. However, in light of the above toxicology information, risk to mammalian wildlife is expected to be minimal to nonexistent.

3) Nontarget Plants

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

4) Nontarget Beneficial Organism Studies

a)Honey Bees

The reviewed honey bee study suggest that at the expected environmental exposure the proposed use of Cry9C protein in corn is not likely to have any measurable deleterious effects on the honey bee (*Apis mellifera)*. The data showed no treatment mortality or behavior change between the dosed and control replicates. The no-observed-effect concentration of 5.8 μg Cry9C protein/L diet (24,000 μg corn pollen/mL) for 8 days indicates that Cry9C protein as expressed in corn pollen showed no toxicity to adult honey bees. The test insects were exposed to a constant dose of pollen for eight days. This is more than the amount that the bees would be expected to consume under field use conditions. As a result, no discernible detrimental effects to honey bees are expected from the proposed uses of the Cry9c producing corn.

The data adequately address potential toxicity concerns for foraging honey bees exposed to Cry9C protein expressed in corn pollen in the field. In addition, since corn is wind pollinated, few honey bees are expected to be exposed.

b) Lady beetle predator:

The reviewed study suggests that corn pollen containing the Cry9C toxin should not cause significant adverse effects to lady bird beetle predators. The data showed no treatment mortality or behavior change between the dosed and control replicates. The LC_{50} of >0.36 μg Cry9C protein/L (1.5g corn pollen/mL), indicates that Cry9C protein as expressed in corn pollen is practically nontoxic to *Hippodamia convergens* as an indicator species for predatory beetles. The test insects were exposed to a dose of active ingredient approximating the amount that would be ingested by the beetles consuming aphids under field conditions. As a result, no discernible beneficial beetle population effects are expected from the proposed uses of the Cry9C producing corn. These data adequately address potential concerns for Cry9C protein expressed in corn to beneficial beetles.

c) Earthworm:

The submitted data demonstrate that at 16.7 X field concentration (EEC) the Cry9C protein endotoxin is practically nontoxic to earthworms. The data adequately address potential concerns for Cry9C protein expressed in corn to beneficial soil invertebrates. The study is scientifically sound and no treatment mortality or behavior change was observed between the dosed and control replicates. The LC_{50} of Cry9C protein for earthworms (*Eisenia fetida*) is > 1.84 mg a.i./kg dry soil (defined as the maximum hazard dose tested) in a 14-day exposure study. The no observed effect concentration is > 1.84 mg a.i./kg dry soil. Given that the amount of Cry9C protein at harvest is 99 g/ac, the expected environmental concentration (EEC) of Cry9C will be 0.11 mg/kg dry soil (15 cm deep). The 1.84 mg test dose represents a level of exposure > 16.7 times the actual contact the earthworm would have under field conditions.

The submitted data show that *Bacillus thuringiensis* subsp. *tolworthi* Cry9C protein has no measurable deleterious effects on earthworms, a representative beneficial soil invertebrate species. This suggests that the proposed uses of the Cry9C protein in corn is not likely to have any measurable population effects on beneficial soil invertebrates.

d) *Collembola*:

The submitted study adequately address potential concerns for Cry9C protein expressed in corn to collembola (*Folsomia candida*) a beneficial soil insect. The study is scientifically sound (core) and no treatment mortality or behavior change was observed between the dosed and control replicates.

There was no toxicity to collembola by chronic exposure to Cry9C from plants or bacteria at any concentration tested. The study showed that at field use rates reproduction of the test insects was not impaired. A slight reduction in fecundity was noted at the highest doses tested (1,637 times the EEC for plant Cry9C and 1.82 million times the EEC for bacterial Cry9C). The only statistically significant effect was a reproduction rate reduction in the 50% bacterial Cry9C treatment (at 5 orders of magnitude higher than the Expected Environmental Concentration). A similar (not statistically significant) effect was noted in the 50% plant Cry9C and plant control replicates.

This study determined that the LD_{50} of *corn plant* Cry9C protein and *bacterial* Cry9C protein to collembola (*Folsomia candida*) over a 28-day exposure period is greater than 50% (by weight) of the diet. The no-effect-level for *corn plant* Cry9C protein was 50% of the diet (equivalent to 180 mg/kg dry soil) . The no-effect-level for *bacterial* Cry9C protein was 5% of the diet (equivalent to 20 gm/kg dry soil). Given that the amount of Cry9C protein at harvest is 99 g/ac, the expected environmental concentration (EEC) of Cry9C will be 0.11 mg/kg dry soil (15 cm deep). The 5% bacterial and the 50% plant Cry9C protein test doses represent a level of exposure $>1.8 \times 10^5$ and 1,637 times the actual contact (EEC) that collembola would have under actual field conditions after harvest.

The reviewed data show that *Bacillus thuringiensis* subsp. *tolworthi* Cry9C corn protein has no measurable deleterious effects on collembola (*Folsomia candida*), a representative beneficial soil insect species. This suggests that the proposed uses of the Cry9C protein in corn are not likely to have any measurable population effects on beneficial soil insects.

e) Host range study

The test organisms were neonate larvae of *Ostrinia nubilalis* (European corn borer), *Helicoverpa zea* (corn earworm), and *Heliothis virescens* (tobacco budworm) and third instar larvae of *Plutella xylostella* (diamondback moth). The Cry9C protein retained the expected activity against all insects tested, regardless of the source (bacterial or corn tissue). The small amount of toxin present in the pollen resulted in mortality rates too low for LC_{50} determinations. The study does, however, show

that there is no apparent change in insect host range as a result of expression of the Cry9C protein in corn.

f) Nontarget Insect Study

This study was not specifically required for the registration of this product. It was conducted voluntarily by the registrant to determine whether *Bt* plant-pesticide maize, expressing truncated *Bt*-derived Cry9C δ-endotoxin, had a significant negative impact on natural nontarget insect populations. The study did provide some useful information on the differences in toxicity to nontarget insect species between chemical insecticides and *Bt*-expressing corn plants, but had some deficiencies. Therefore, the study will be considered supplemental. Since this was not a required study, it will not have to be repeated.

There was no consistent pattern of differences in abundance of predatory insects on the Cry9C versus the control corn plots throughout the growing season. Trapping height had no significant impact on the number of predators captured. Testing of larger plot sizes would, however, produce more significant results. Therefore it is recommended that the beneficial insect monitoring should continue into the first few years of commercial use of the transgenic corn crops to confirm the small plot "no effects" findings.

c. Risk Characterization for Aquatic Animals

1) Fish

The requirement for a static renewal toxicity study has been waived based on a lack of exposure of fish to the *Bt* endotoxin Cry9C protein produced in corn. PGS has determined that an analysis using ELISA indicated that Cry9C was not detectable in fish diet made using corn containing the *Bt* Cry9C δ-endotoxin. Cry9C activity was shown to be destroyed following the extrusion process utilized in a typical fish food manufacturing process. Therefore, fish eating a food mix made from corn containing the *Bt* δ-endotoxin would not be exposed to detectable active *Bt* δ-endotoxin protein. Thus little or no exposure to cultured fish is expected from commercial preparations of fish food, and further fish studies are waived.

2) Aquatic Invertebrates

A 48-hour static renewal toxicity study with *Daphnia magna* showed no treatment mortality or behavior change between the dosed and control replicates. Since the test and control solutions appeared cloudy with yellow particles in suspension and settled on the bottom of the test chambers, the study is graded supplemental since the amount of pollen that the Daphnia were exposed to could not be determined. However, the amount Daphnia were exposed to is considered far greater than the

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EEC and no adverse affects were noted. The data adequately address potential aquatic toxicity concerns for Cry9C protein expressed in corn and no additional studies should be needed in order to complete the aquatic invertebrate risk assessment. The data suggest that at the expected environmental concentration of corn pollen from the proposed use of Cry9C protein in corn is not likely to have any measurable population effects on aquatic invertebrates.

3) Estuarine and Marine Animals

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

d. Impacts on Endangered Species

A Biological Opinion was issued on December 18, 1986, concerning the possible effect of foliar spray of *Bacillus thuringiensis* subsp. *kurstaki* (*Bt*) on threatened and endangered species. Based on the difference in exposure scenarios between foliar *Bt* spray and *Bt* delta endotoxin expressed in corn plants, EPA believes that the Biological Opinion is not applicable and that reinitiation of consultation is not required.

The primary route of exposure to foliar *Bt* sprays is through either direct application to the crop or as a result of drift from spray or aerial applications. In comparison, the primary route of exposure to *Bt* delta endotoxin in corn is through ingestion of corn tissue. There are no reports of threatened or endangered species feeding on corn plants, thus such species would not be exposed to corn tissue containing the Cry9C delta endotoxin. Corn is widely grown, and above ground feeding damage is easily observed on corn plants due to its morphology and cultural practices. Since Cry9C corn pollen have shown no toxicity to the animal, insect and invertebrate species tested, hazard to soil and aquatic organisms is not expected to occur.

Another possible route of exposure is from corn pollen containing the delta-endotoxin that can drift from corn fields. As discussed previously in this section, the applicant has submitted adequate data for representative species to substantiate that the Cry9C protein is practically nontoxic.

In addition, EPA does not expect that any threatened or endangered species will be affected by outcrossing to wild relatives or by competition with such entities. Hybrid corn cannot exist in the wild, nor are there wild relatives that can interbreed with corn in the United States.
Because EPA expects that threatened or endangered Lepidopteran insects and other species will not be exposed to *Bt* delta endotoxin, and because the most probable exposure scenario does not appear to affect listed species, EPA believes that this action will have no effect on any threatened or endangered species.

D. INSECT RESISTANCE MANAGEMENT

Table # 6 : Insect Resistance Management

"Insect Resistance Management Plan: Bt Cry9C Corn" dated April 1, 1997, MRID # 442581-16; and "Updated Insect Resistance Management Plan: *Bacillus thuringiensis* Cry9C Corn" dated January 26, 1998, MRID # 445042-01.	442581-16, 445042-01

The PGS proposal addresses the major elements of the an Insect Resistance Management (IRM) program as defined by the Agency's guidelines (approved by the March 1, 1995 Science Advisory Panel (SAP) on plant-pesticides). The following resistance management elements are addressed in the plan: primary target pest biology, secondary pest biology, pest susceptibility, potential IRM strategies, monitoring of pest populations and remedial action strategy, IPM practices and the impact of Cry9C corn, communication and education, Bt binding sites/cross resistance potential and future transgenic advances including alternate modes of action. However, some elements lack specific detail and will need to be clarified by the company. Further modifications will have to be made in other areas of the IRM plan to effectively manage pest resistance in Cry9C corn beyond the length of this registration.

The PGS plan provides information regarding the target pests of Cry9C corn, although further information is still needed for these pests. The Cry9C toxin has been shown to be highly lethal to European corn borer (ECB, *Ostrinia nubilalis* Hübner), the primary pest of corn throughout North America. PGS is currently evaluating the susceptibility of the southwestern corn borer (SWCB, *Diatraea grandiosella* Dyar) to Cry9C. Other corn pests, including the black cutworm (BCW, *Agrotis ipsilon* Hufnagel), and the southern corn stalk borer (SCSB, *Diatraea crambidoides* Grote) should also be examined for susceptibility to Cry9C. There are also strong indications that Cry9C has little if any toxic effects on the corn earworm (CEW, *Helicoverpa zea* Boddie), although further research should be carried out to determine if any fitness costs (a possible source of selection pressure) are involved with CEW and Cry9C. CEW is of concern in Southern cotton growing regions, since it typically moves from browning corn fields to cotton during the season. At this time, it appears the risk of CEW resistance developing to Cry9C is minimal. If fitness costs prove insignificant, CEW resistance does not appear to be a concern for Cry9C Bt corn and no restrictions will be recommended in Southern cotton growing regions.

Most experts in the field agree that refugia in combination with a high expression of toxin represents the best available strategy to mitigate the threat of resistance. The PGS proposal indicates that the company believes that Cry9C corn expresses a high dose of toxin. Submitted data appears to verify this in all plant tissues except pollen. However, it is recommended that the reported "high dose" be

verified using the procedures outlined by the recent February 1998 SAP subpanel. PGS does not provide a specific refuge strategy in their proposal. This should be done throughout the growing season to cover all generations of Cry9C susceptible corn pests. Given the consensus view of experts (including the SAP) that refuge is an absolute requirement for IRM, it is strongly advisable that the USDA NC-205 guidelines (25% untreated or 40% treated non Bt corn refuge in close proximity to the Bt crop) or a substantially similar refuge strategy be adopted for Cry9C and all Bt corn products. Based upon current scientific evidence, the refuge should be deployed within 1500-2000 feet of the Bt crop.

Resistance monitoring is a crucial aspect of any IRM program. PGS plans to monitor for resistance in counties that contain at least 25% Cry9C corn and in response to reports of field failure. This proposal is a limited approach that may overlook resistance developing in less densely planted areas not routinely sampled. A more thorough resistance monitoring plan that encompasses the entire distribution region of Cry9C corn will have to be developed. Additionally, PGS does not specify what resistance monitoring techniques will be employed. It is recommended that PGS collect baseline susceptibility data for the target pests of Cry9C corn and also develop a discriminating dose assay and F_2 screen. While these assays are being developed, PGS should use LC_{50} diet bioassays and leaf damage assays to monitor for resistance (techniques currently mandated by EPA for other Bt corn products).

In cases of confirmed resistance, PGS proposes a remedial action plan that, if implemented, would be the most aggressive strategy of all currently registered Bt corn products. This plan calls for the immediate suspension of seed sales in the affected counties once resistance has been confirmed. This is a logical first step that should be required for all Bt corn products.

Cry9C corn is the first Bt corn product submitted for registration that does not contain either the CryIA(b) or CryIA(c) toxins. In addition, there are no other currently registered Bt pesticide products, either microbial or transgenic, that contain Cry9C. Cry9C has been shown to bind to specific insect midgut receptors that differ from the binding receptors of CryIA(b) or CryIA(c). Due to these separate binding sites, there should be a relatively low potential for cross resistance between Cry9C and CryIA(b) or CryIA(c). Therefore, as a Bt toxin with a different mode of action, Cry9C may have added value in a transgenic crop system presently inundated solely with CryIA toxins and in a future multiple toxin deployment (pyramid) strategy. However, further work should be done to explore the potential of cross resistance with other Bt toxins.

Cry9C corn should be fully compatabile with current Integrated Pest Mangement (IPM) practices for corn. The PGS proposal indicates that grower education will be a large part of their IRM plan, although the specific information that will be conveyed to growers has not been described. Growers are at the interface between IRM and the insect pests and their education is critical to the success of any IRM plan. PGS should detail specific IRM recommendations regarding refuge, IPM, monitoring, reporting of unexpected damage, and any local or regional considerations.

For the limited 120,000 acre registration, the following limitations would be sufficient to mitigate the risk of resistance developing to the Cry9C protein.

1) Refuge requirements reflecting the NC-205 recommendations are required for this product. Most experts in the field, the February 1998 SAP, and the Agency agree that the deployment of refugia must be a requirement of any IRM program. Although there is still uncertainty and disagreement among scientists as to the optimal refuge design for Bt corn, it is recommended that the USDA NC-205 guidelines be mandated for Cry9C corn. These guidelines reflect the current knowledge base among USDA, academic, industry, and EPA scientists. Specifically, a 25% unsprayed or 40% sprayed non-Bt corn structured refuge in close proximity to the Bt crop is recommended. Based upon additional research, the refuge should be established within 1500-2000 feet of the Bt crop.

2) PGS must provide specific information through their technical bulletins, brochures, product labels, and educational presentations so that growers have the necessary tools to successfully implement an IRM plan. A World Wide Web site on the internet would be a practical way to provide specific resistance management information. Included in this IRM information should be instructions on the appropriate use of the Bt plant-pesticides in a resistance management program, compatibility with existing Integrated Pest Management (IPM) programs, refuge deployment and management (including IPM options), monitoring, reporting of unusual pest damage, and any local and regional IRM considerations. The success of any IRM program will ultimately depend on growers who have the knowledge and tools to understand the problem of resistance and the steps that can be taken to combat it.

3) PGS must maintain a (confidential) database to track sales by units and location of Cry9C corn on a state and county-by-county basis. This material should be submitted annually (by January 31 of the year following each growing season) to the Agency on a Confidential Business Information (CBI) basis. As part of this report, PGS should provide an estimate of the acreage for Cry9C corn within each state.

The following additional data and terms will be necessary to support a more broad use of this plant-pesticide product.

1) Research must be conducted to expand the knowledge base for the following pests that are or may be affected by Cry9C corn: European corn borer (ECB, *Ostrinia nubilalis* Hübner), southwestern corn borer (SWCB, *Diatraea grandiosella* Dyar), and black cutworm (BCW, *Agrotis ipsilon* Hufnagel). The February 1998 SAP meeting identified research needs for adult movement, pre- and post-mating dispersal, mating behavior, ovipositional patterns, fitness, and larval movement. This information is critical to the optimal design, placement, and evaluation of refugia.

2) PGS must conduct a susceptibility study with the corn earworm (CEW, *Helicoverpa zea* Boddie) to determine if there are any fitness costs associated with exposure to Cry9C toxin. Submitted research has clearly indicated that there is little or no mortality of CEW associated with Cry9C. However, fitness costs, such as growth inhibition and delayed developmental time, may be an indication of toxicity and also a source of selection pressure. CEW is a pest of great concern in cotton growing areas since it is a pest of both corn and cotton and frequently moves from corn to cotton during the season. This creates the potential for multiple exposure to different Bt toxins between the two crops. Because of these resistance management concerns with CEW, a thorough investigation of all possible sources of resistance selection (including fitness costs) should be undertaken.

3) PGS must examine the Cry9C susceptibility of primary and secondary pests of corn including the SWCB, BCW, and southern corn stalk borer (SCSB, *Diatraea crambidoides* Grote). These insects have not yet been evaluated for susceptibility to the Cry9C toxin. Although these pests will not initially appear on the Cry9C corn label, they may still be affected by the Cry9C toxin and have an impact on resistance management. PGS has indicated in subsequent communications that susceptibility studies for SWCB have been planned and that SWCB and BCW may be added to the label in the future.

4) PGS must verify the high dose claimed for Cry9C corn using the guidelines established by the February 1998 SAP. The presence of a high dose of Bt toxin in transgenic crops is considered vital to the high dose/refuge strategy. Data cited by PGS seem to indicate that there is a high dose of toxin expressed in all Cry9C corn tissues except pollen, relative to the LC_{50} of first instar ECB larvae. However, given the importance of a "high dose," this claim should be further verified using the SAP guidelines. "High dose" was defined as 25 times the amount of toxin necessary to kill susceptible larvae. Five techniques were identified at the SAP meeting, of which at least two should be used to confirm a high dose. These were: 1) Serial dilution bioassays with artificial diet incorporating Bt plant tissue and non-Bt plant tissue of the same cultivar (control); 2) Bioassays with Bt plants that express 25 times less toxin than commercial Bt plants of the same cultivar; 3) Surveys of large numbers of commercial Bt plants in the field--verify that the $LD_{99.99}$ for susceptibles is present and to also assure that 95% of heterozygotes will be killed; 4) Controlled infestations on the cultivar with insects of a known LC_{50}; 5) Bioassays on Bt plants with older instar larvae that are 25 times less susceptible–verify that 95% or greater are killed by the cultivar. The high dose should be verified throughout the growing season (not just at one point during the season) to cover all generations of corn pests.

5) PGS must continue to conduct research on refuge deployment. Suggested areas of focus include the evaluation of: 1) in-field block or strip refuges; 2) external non-Bt corn refuges; and 3) alternate host crops as refuge.

6) PGS must implement a resistance monitoring program that is not tied to a specific sales threshold. PGS proposes to monitor for resistance to Cry9C only in counties in which Cry9C corn accounts for at least 25% of the total corn grown. However, since the initial market penetration may be relatively low in the first few years of Cry9C corn availability, this type of monitoring plan may not provide an adequate sampling of the Cry9C corn distribution. Instead, a plan is recommended that will focus on both counties with a relatively high Cry9C corn distribution (not necessarily 25%) and also at a sufficient number of lower density sites to adequately represent the entire distribution region. The resistance monitoring plan should encompass all Cry9C susceptible pests, especially labeled pests. Resistance monitoring should be required in areas where the target pests are known to regularly overwinter. For ECB, this includes most of the United States, but for more migratory pests such as SWCB, monitoring can be focused on Southern overwintering sites (e.g. Texas).

7) PGS must utilize a specific resistance monitoring program for all the target pests affected by Cry9C corn. Along these lines, PGS should collect baseline susceptibility data for the target pests of Cry9C corn, which can be helpful in documenting the extent and distribution of resistance. In addition, PGS must work to develop both a discriminating dose assay (to detect dominant resistance alleles) and a F_2 screen (to detect recessive resistance alleles) for each target pest. Current Agency requirements for other Bt corn products mandate the development of baseline susceptibility data and discriminating dose bioassays. These techniques may take time and research to develop. Until such time as these assays are available, PGS should utilize LC_{50} diet bioassays and leaf damage assays, which have also been mandated for previous Bt corn registrations. The use of in-field monitoring for pest damage and sentinel plots may also provide practical means of detecting resistance alleles. In their proposal, PGS does not clearly indicate the techniques, other than investigating reports of unexpected damage, that will be undertaken to monitor for resistance. Detecting shifts in the frequency of resistance genes through resistance monitoring can be an aggressive method to detect the onset of resistance before widespread crop failure occurs. As such, resistance monitoring is critical to the success of an IRM plan.

8) PGS must fully investigate the potential for Cry9C cross resistance with CryIF, CryIIA, and CryIC for all of the pests of corn that may be susceptible to Cry9C (ECB, SWCB, BCW, possibly SCSB). CryIF shares a binding site with CryIA(a), CryIA(b), and CryIA(c), while CryIIA is found in some foliar Bt products and has shown broad cross resistance potential to CryIA in some cases. In addition, Cry9C is known to partially share a binding site with CryIC (a component of some microbial Bt products) in the beet armyworm (BAW, *Spodoptera exigua* Hübner) and ECB. Cross resistance, through shared binding sites or other mechanisms, is an area of major concern for resistance management. The PGS plan cites data which indicates that Cry9C does not share a binding site with CryIA(b) or CryIA(c) (the Bt toxins present in all other registered Bt corn products) in the diamondback moth (DBM, *Plutella xylostella* L.) and ECB. Due to these separate binding sites, there should be a relatively low potential for cross resistance between Cry9C and CryIA(b) or CryIA(c).

9) PGS must provide to the Agency on annual basis (by Januray 31 of the year following each growing season) a detailed (complete with study summaries, methods and protocols, and results) report of ongoing resistance management activities research. This report should be on a non-CBI basis.

IV. Risk Management Decision

BPPD believes that a section 3(c)(5) registration that is limited in scope and duration is appropriate in this situation. This registration will be limited as to the duration of the registration, the acreage of corn plants grown, and subsequent harvesting and processing of the resulting crop. BPPD in recommending a finding of no unreasonable adverse effects, is relying in part upon the limitations set in the limited registration. In addition, the registration stipulates that the company acquiring the registration is liable for the actions of their customers in terms of meeting the terms and limitations of the registrations. Data requirements for granting this limited plant propagation registration under section 3(c)(5) of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) have been met. The data have been reviewed and BPPD forsees no unreasonable adverse effects from the use of this product if it is used pursuant to the limitations of the registration.

B. REGULATORY POSITION

1. Registration

1. The registration will automatically expire on midnight May 30, 1999. After this registration has expired, no field corn seed that contain the pesticide product may be sold or planted. However, harvesting of such corn planted prior to May 30, 1999 is permissible subject to the terms of this registration. Plant Genetic Systems (America) is liable for the actions of its customers in regard to meeting the terms and limitations of this registration

2. The registration is for field corn to be used only in animal feed, industrial non-food uses such as ethanol production, and seed increase. In addition, any corn grown within 660 feet of Cry9C corn must also be limited to use in animal feed and industrial non-food uses such as ethanol production. The acreage of corn planted may not exceed 109,000 acres for the animal feed and industrial uses and 11,000 acres for seed increase.

3. Plant Genetic Systems must require that growers follow the USDA NC-205 guidelines for refuge for all Cry9C corn. These guidelines reflect the current knowledge base among USDA, academic, industry, and EPA scientists. Specifically, a 25% unsprayed or 40% sprayed non-Bt corn structured refuge in close proximity to the Bt crop is required. The refuge must be established within 1500-2000 feet of the Bt crop. Any insecticide treatment cannot include Bt sprayble products.

4. Plant Genetic Systems must provide and indicate how it will provide specific information through their technical bulletins, brochures, product labels, and educational presentations so that growers have the necessary tools to successfully implement an IRM plan. A World Wide Web site on the internet would be a practical way to provide

specific resistance management information. Included in this IRM information should be instructions on the appropriate use of the Bt plant-pesticides in a resistance management program, compatibility with existing Integrated Pest Management (IPM) programs, refuge deployment and management (including IPM options), monitoring, reporting of unusual pest damage, and any local and regional IRM considerations. The success of any IRM program will ultimately depend on growers who have the knowledge and tools to understand the problem of resistance and the steps that can be taken to combat it.

5. Plant Genetic Systems must maintain a (confidential) database to track sales by units and location of Cry9C corn on a state and county-by-county basis. This material should be submitted annually (by January 31 of the year following each growing season) to the Agency on a Confidential Business Information (CBI) basis. As part of this report, Plant Genetic Systems must provide an estimate of the acreage for Cry9C corn within each state.

2. Tolerance

Table #5: Table of Tolerances for *Bacillus thuringiensis* subspecies tolworthi Cry9C protein genetic material necessary for its production in all plants.

Raw or Processed Commodity	Exemption from the Requirement of a Tolerance	Comments: Official Date: 8/2/96
Tolerance Exemption Listed under 40 CFR § 180.1192		
Sec. 180.1192 Bacillus thuringiensis subspecies tolworthi Cry9C protein and the genetic material necessary for its production in corn; exemption from the requirement of a tolerance. The plant-pesticide Bacillus thuringiensis subspecies tolworthi Cry9C protein and the genetic material necessary for its production in corn is exempted from the requirement of a tolerance for residues, only in corn used for feed; as well as in meat, poultry, milk, or eggs resulting from animals fed such feed.		

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3. CODEX Harmonization

This active ingredient is not currently listed in CODEX.

4. Risk Mitigation

The terms and conditions of the registration mitigate the risk of allergenicity and insect resistance.

5. Endangered Species Statement

A Biological Opinion was issued on December 18, 1986, concerning the possible effect of foliar spray of *Bacillus thuringiensis* subsp. *kurstaki* (*Bt*) on threatened and endangered species. Based on the difference in exposure scenarios between foliar *Bt* spray and *Bt* delta endotoxin expressed in corn plants, EPA believes that the Biological Opinion is not applicable and that reinitiation of consultation is not required.

The primary route of exposure to foliar *Bt* sprays is through either direct application to the crop or as a result of drift from spray or aerial applications. In comparison, the primary route of exposure to *Bt* delta endotoxin in corn is through ingestion of corn tissue. There are no reports of threatened or endangered species feeding on corn plants, thus such species would not be exposed to corn tissue containing the Cry9C delta endotoxin. Corn is widely grown, and above ground feeding damage is easily observed on corn plants due to its morphology and cultural practices. Since Cry9C corn pollen have shown no toxicity to the animal, insect and invertebrate species tested, hazard to soil and aquatic organisms is not expected to occur.

Another possible route of exposure is from corn pollen containing the delta-endotoxin that can drift from corn fields. As discussed previously in this section, the applicant has submitted adequate data for representative species to substantiate that the Cry9C protein is practically nontoxic.

In addition, EPA does not expect that any threatened or endangered species will be affected by outcrossing to wild relatives or by competition with such entities. Hybrid corn cannot exist in the wild, nor are there wild relatives that can interbreed with corn in the United States.
Because EPA expects that threatened or endangered Lepidopteran insects and other species will not be exposed to *Bt* delta endotoxin, and because the most probable exposure scenario does not appear to affect listed species, EPA believes that this action will have no effect on any threatened or endangered species.

C. LABELING RATIONALE

It is the Agency's position that the labeling for products containing Bacillus thuringiensis subspecies tolworthi Cry9C protein and the genetic material necessary for its production in corn

complies with the current pesticide labeling requirements and contain the following label elements.

Product name
Ingredient Statement
Signal Word
Precautionary Statements
Registration Number
Establishment Number
"Keep Out of Reach of Children" Statement
Text for Informational Material to Accompany Seed in Commerce. This must include insect resistance management and distribution limitation information as well as the fact that the corn contains Bt Cry9C.

V. Actions Required by Registrants

Reports of incidences of adverse effects to humans or domestic animals under FIFRA, Section 6(a)2 including incidents of hypersensitivity and resistance.