US Environmental Protection Agency
Office of Pesticide Programs

BIOPESTICIDE REGISTRATION ACTION DOCUMENT
Bacillus thuringiensis Cry1F Corn

August 2004

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

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I. Overview

A. Cry1F Event TC1507 (Event DAS-01507-1)

OPP Chemical Code: 006481

Pesticide Name: *Bacillus thuringiensis* subspecies Cry1F Protein and the genetic material necessary for its production (Plasmid Insert PHI 8999) in corn

Trade and Other Names: Herculex™ I Insect Protection, Pioneer Brand Seed Corn with Herculex™ I

Applicants: 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

Uses: Full Commercial Use in Field Corn

Regulatory History:

Mycogen Seeds (c/o Dow Agrosciences LLC) and Pioneer Hi-Bred International, Inc. a Dupont company, registered a *Bacillus thuringiensis* subspecies Cry1F protein and the genetic material necessary for its production (plasmid insert PHI 8999) in corn. Cry1F maize line 1507 expresses a modified (synthetic, less than full length) form of the *cry*1F gene, originally isolated from *Bacillus thuringiensis aizawai*, and the phosphinothricin-N-acetyl transferase (*pat*) gene which is derived from *Streptomyces viridochromogenes*. These registrations were granted on May 18, 2001 and assigned EPA Registration Numbers 68467-2 and 29964-3, respectively. The expressed Cry1F protein effectively controls certain lepidopteran insect larvae including European corn borer (ECB; *Ostrinia nubilalis*), southwestern corn borer (SWCB; *Diatraea grandiosella*), fall armyworm (FAW; *Spodoptera frugiperda*) and black cutworm (*Agrostis ipsilon*). A summary of the Agency's risk and benefits assessment for Cry1F maize line 1507 corn is found in the 2001 Cry1F Biopesticides Registration Action Document (EPA, 2001a) and updated in the Agency's Bt Crops Reassessment (EPA, 2001b). An exemption from the requirement of a tolerance for *Bacillus thuringiensis* Cry1F protein and the genetic material necessary for its production in corn (including corn with plasmid insert PHI 8999) was granted on June 6, 2001 and is found at 40 CFR 180.1217. As part of the Agency's reassessment of Bt crops completed in 2001 (EPA, 2001b), the Cry1F event TC1507 registrations (68467-2 and 29964-3) were extended from September 30, 2001 to October 15, 2008.

B. Cry1F Event **DAS-06275-8**

OPP Chemical Code: 006491

Pesticide Name: *Bacillus thuringiensis* var. *aizawai* strain PS811 Cry1F insecticidal crystal protein and the genetic material necessary for its production (plasmid insert PHP12537) in Event DAS-06275-8 corn

Trade and Other Names: Mycogen Brand *B.t.* Cry1F Event DAS-06275-8 Corn

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

Uses: Full Commercial Use in Field Corn

Regulatory History:

Mycogen Seeds (c/o Dow Agrosciences LLC) applied to register a maize-optimized genetically engineered *Bacillus thuringiensis* (B.t.) (from the insert of plasmid PHP12537) corn plant-incorporated protectant that contains a synthetic *cry*1F gene and expresses the Cry1F protein as well as phosphinothrin acetyl transferase (*bar* gene isolated from *Streptomyces hygroscopicus*). The Cry1F Event DAS-06275-8 protein, like the Cry1F Event TC1507 protein, protects the corn from certain lepidopteran insect larvae including European corn borer (ECB; *Ostrinia nubilalis*), southwestern corn borer (SWCB; *Diatraea grandiosella*), fall armyworm (FAW; *Spodoptera frugiperda*) and black cutworm (*Agrostis ipsilon*). The pesticide active ingredient is known as *Bacillus thuringiensis* Cry1F protein and the genetic material necessary for its production in corn. An exemption from the requirement of a tolerance for *Bacillus thuringiensis* Cry1F protein and the genetic material necessary for its production in corn (non-Event specific) was granted on June 6, 2001 and is found at 40 CFR 180.1217. An Experimental Use Permit, 68467-EUP-4, was originally granted on April 10, 2002 for one year. An extension/amendment to this EUP was effective from April 11, 2003 to March 31, 2004.

II. Science Assessment

The classifications that are found for each data submission are assigned by the EPA science reviewer and are an indication of the usefulness of the information contained in the documents and meets the intent of the test guidelines. A rating of "ACCEPTABLE" indicates the data is useful for risk assessment, is scientifically valid and has been satisfactorily performed according to accepted EPA guidelines or other justified criteria. A "SUPPLEMENTAL" rating indicates the data provide some

information that can be useful for risk assessment. However, "SUPPLEMENTAL" studies may either have certain aspects not adequately described to be scientifically acceptable (SUPPLEMENTAL. UPGRADEABLE) or have not been done to fulfill a specific EPA guideline requirement. If a study is rated as "SUPPLEMENTAL UPGRADEABLE" there is always an indication of what is lacking or what can be provided to change the rating to "ACCEPTABLE". If there is simply a "SUPPLEMENTAL" rating, the reviewer will often state that the study is not required by current EPA guidelines or does not need to be reclassified as "ACCEPTABLE". An "UNACCEPTABLE" rating indicates that the study was scientifically compromised and cannot be used for risk assessment purposes.

A. PRODUCT CHARACTERIZATION

1. CRY1F Event TC1507 (EPA, 2001a and b)

A corn line of Pioneer Hi-Bred International and Dow Agrosciences / Mycogen was biolistically transformed with a linear *Pme*I fragment from plasmid pP8999 to produce line 1507. This plasmid contains genes *cry1F*, *pat* and *kan*^{r} encoding the delta-endotoxin from *Bacillus thuringiensis* var. *aizawai* PS811, phosphinothricin acetyl transferase, and resistance to the antibiotic kanamycin, respectively. The *Pme*I fragment (6235 bp) derived from this plasmid was purified after plasmid digestion and used in the transformation process to eliminate the *kan*^{r} antibiotic resistance gene. The Cry1F protein expressed in transformed maize lines is a modified (synthetic, less than full length) form as compared to that from the bacterial isolate from which it is derived. This insecticidal protein confers resistance to the European corn borer (*Ostrinia nubilalis*) and feeding damage is significantly reduced or eliminated following expression of this gene in corn line 1507. Expression of *cry1F* is under the control of the maize polyubiquitin promoter in line 1507. The CaMV 35S promoter controls expression of the *pat* gene in this construct. The *pat* gene from *Streptomyces viridochromogenes* confers resistance to the herbicide glufosinate in corn lines accumulating this protein. Hybridization patterns indicate that one full length copy each of the *cry1F* and *pat* genes was integrated into the genome of line 1507 and that no *kan*^{r} DNA was integrated. This suggests that one *Pme*I fragment from pP8999 integrated into the maize genome. In addition, there are one or two partial copies of the *cry*1F gene integrated into the genome which are most likely non-functional based upon the size of the fragments detected.

Study	*Result*	*MRID #*
Quantitative ELISA analysis of Cry1F expression levels in maize MPS inbreds and hybrid lines 1360, 1365, 1366, and 1369. (Interim report)	Maize plants (hybrids) from two locations grown under standard agronomic practices of the Midwestern Corn Belt were analyzed by ELISA for Cry1F protein content. The youngest leaf of expanding whorls at the V9 stage was collected from five plants per entry. Values of Cry1F protein for all four hybrids were similar, ranging from 1.52 to 2.63 pg/cg dry weight. Control hybrid A_M was negative for Cry1F as determined by ELISA. CLASSIFICATION: ACCEPTABLE	447148-04
Product characterization data for Bacillus thuringiensis var. aizawai Cry1F as expressed in maize.	A modified (synthetic, less than full-length) form of the cry1Fa2 gene and the phosphinothricin acetyl transferase (pat) gene were inserted into maize plants by microprojectile bombardment. Three transformation events resulting from microprojectile bombardment will be evaluated under the proposed EUP: TC 1360, TC 1362 and TC 1507. Plants were analyzed for Cry1F by ELISA and PAT by application of glufosinate herbicide. Using a chi square analysis with a 95 % confidence interval, the expected Mendelian ratio of 1:1 was observed for both first and second generations for five inbreds with one exception; first generation TC 1632. Event TC 1507 has been analyzed for only the first generation and ratios (1:1) were as expected. CLASSIFICATION: SUPPLEMENTARY. The registrant should clarify the source of the ubiquitin exon and intron as being from the ubiquitin gene and not the promoter region. A determination of expression of the ubiquitin exon sequence is also needed and whether it alters the sequence of Cry1F.	447148-01
Supplement to MRID 447148-01: Supplemental Data – Product Characterization Data for Bacillus thuringiensis var. aizawai Cry1F Insect Control Protein as Expressed in Maize	This submission represents a clarification of nomenclature as presented in a previous submission and review. Labeling (in a previous submission) of the Ubi DNA fragment on the plasmid map should have indicated that it includes the Ubi ZM promoter and the first exon and intron of the Ubi ZM gene. The Ubi exon and intron are included in this construct (PHI8999), however, they have no effect on the structure of the Cry1F product, only on the expression of the gene. Exon 1 contains no ATG start site for translation. A translation initiation sequence (Kozak consensus sequence) situated just upstream from the start site (first translated ATG) drives translation of the mature, spliced mRNA. CLASSIFICATION: ACCEPTABLE	450201-17
Characterization of gene inserts – Bacillus thuringiensis var. aizawai Cry1F insect control protein as expressed in maize.	The integration pattern of cry1F and pat genes introduced into event TC 1360 was analyzed by Southern blotting. Within the Southern analysis, two types of digests are employed to determine the complexity of DNA integration into the maize genome and to determine the copy number of integrated transgenes. Analysis of four of the progeny from event TC 1360 revealed the presence of two bands hybridizing to the cry1F probe; both bands appeared to hybridize with similar intensity. Hybridization to internal controls on the blot gave an indication of single copy integration and certainly no more than two copies of the insert integrated into the maize genome. When control plant DNA was probed, no hybridization was noted. TC 1360 and control DNA probed with the (plasmid insert PHP12537) kan^r gene indicated no hybridization within these samples. CLASSIFICATION: ACCEPTABLE	447148-02

Study	*Result*	*MRID #*
Characterization of inserted genes in Cry1F maize line 1507	A modified (synthetic, less than full-length) form of the cry1F 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 NheI or HindIII 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. It is not possible with this technique, however, to discern the functionality of probed sequences. No kan^r DNA was introduced into line 1507 during transformation, as indicated by the lack of signal when 1507 genomic DNA was probed with the kan^r gene. There was no hybridization signal when the non-transformed maize line 13-1 was probed with pat or cry1F or kan^r. CLASSIFICATION: ACCEPTABLE	450201-02
Characterization of expressed Cry1F protein in maize tissues (pollen, grain, grain-containing feed, and purified maize-expressed Cry1F protein) and microbial expressed Cry1F delta endotoxin by biological and biochemical procedures.	Cry1F protein from 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. Control maize tissues were used to prepare comparable samples. Pollen from line 1507 contained Cry1F at 31 to 33 ng / mg pollen, while no Cry1F protein was detected in pollen from non-Cry1F plants. The purified maize-expressed Cry1F test substance was approximately 32 ng / mL extract. The comparable extract from non-Cry1F maize did not show any detectable Cry1F protein; the limit of detection (LOD) was 0.04 ng / mg sample. Coomassie stained gels indicated similar profiles for both control maize and Cry1F maize samples following SDS-PAGE. Antibodies directed against Cry1F detected this protein (64 kDa) in the Cry1F maize grain samples while there was no indication of any Cry1F protein in the control samples of grain. Pollen, maize-expressed Cry1F and microbially derived Cry1F were all active against the European Corn Borer larvae at the times tested. For the Tobacco Budworm larval bioassay, substances tested included maize grain, maize grain derived fish feed, and maize grain derived quail feed. Samples containing Cry1F maize grain and quail feed made from this grain had identical amounts of Cry1F protein based upon the GI_{50}s calculated. Comparison of control and Cry1F fish feed over four separate bioassays indicated that there was no statistical difference ($p = 0.05$) based upon ANOVA. Preparation of the fish feed sample reduced the biological activity of the Cry1F protein below sensitivity for the assay. CLASSIFICATION: ACCEPTABLE	450201-03
Quantitative ELISA analysis of Cry1F and PAT expression levels in compositional analysis of maize inbred and hybrid lines 1362 and 1507	Protein expression values indicated substantial variability in protein levels for Cry1F in the tissues sampled. No definitive conclusions could be reached from the data presented when comparing levels of Cry1F in hybrid 1507 and inbred 1507 when examining pollen, silk, stalk, leaf, grain, whole plant and senescent whole plant samples. Since these hybrids and inbreds were grown in areas of Chile with similar climatic extremes to the maize growing areas of the U.S., it is anticipated that these values will represent those to be expected in the U.S. cornbelt. PAT expression was also not readily distinguishable when comparing inbred and hybrid expression	450201-04

Study	*Result*	*MRID #*
	values. The inability to detect PAT protein in the majority of samples, except leaf, is somewhat puzzling in that the plants demonstrated clear glufosinate tolerance at all field sites. Given the generally strong, non-tissue specific expression levels typically associated with the CaMV 35S promoter (driving pat expression), it is not readily apparent why more PAT protein was not detected in more samples. Its presence in leaf tissue was expected, however, the reason for the absence in many of these samples is less than clear. CLASSIFICATION: ACCEPTABLE	
Cry1F Lateral Flow Test Kit Procedure for Analyzing Cry1F Corn Grain	A double antibody sandwich test was developed to detect the Cry1F protein in homogenized maize grain samples using a rapid test method. A double antibody sandwich technique is used in the Lateral Flow Test Kit for Cry1F. Antibodies raised against the Cry1F protein are incorporated into the Lateral Flow test strip and coupled to a color reagent. When in contact with Cry1F protein, the antibodies bind Cry1F and a sandwich is formed, however, not all of the antibodies are coupled to the color reagent. The test strips contain two zones wherein capture of color reagent or antibodies can occur. One zone captures bound Cry1F and the other captures color reagent. Both zones display a reddish color when protein-antibody sandwich and / or unreacted color reagent are captured. When only one line (control) line is present, a negative sample is indicated, while the presence of two lines indicates the presence of Cry1F. The Cry1F Lateral Flow Test Kit accurately detected Cry1F protein in 30 of 30 corn kernels from Cry1F maize and indicated negative reactions for the 30 control maize kernels. This finding demonstrates the utility of using the Cry1F Lateral Flow Test Kit for detection of Cry1F protein in maize grain samples. This kit allows for a rapid qualitative determination of the presence of Cry1F protein. CLASSIFICATION: ACCEPTABLE	452793-01
Method Validation Report for the Determination of Cry1F Delta-endotoxin Protein in Corn Grain by Enzyme Linked Immunosorbent Assay	The results of this assay validation indicate that the ELISA based assay was suitable for the analysis of Cry1F as found in corn grain. Average recoveries from samples spiked with Cry1F protein (truncated microbial form) were between 67 and 107%. Extractions from known Cry1F corn grain samples demonstrated that a sample as small as 50 mg could be properly extracted and quantified. CLASSIFICATION: ACCEPTABLE	452793-02
Thermolability of Cry1F (truncated) Delta-Endotoxin	The Cry1F test substance was prepared in 10 mM potassium phosphate buffer (pH 7.5) and placed into a water bath at either 60, 75 or 90 °C for 30 minutes, or into the refrigerator at 4 °C. Application of treated Cry1F to the surface of an insect diet and measurement of growth inhibition of neonate tobacco budworm larvae, indicated that the Cry1F protein was labile to heat at and above 75 °C. CLASSIFICATION: ACCEPTABLE	452748-01

Study	*Result*	*MRID #*
Compositional Analysis of Maize MPS Hybrid Line 1507	Protein and nutritional parameters were measured in grain and whole plant samples of hybrid 1507 (expressing Cry1F) and a genetically similar control hybrid, both grown at 4 locations in Chile. Fatty acids, ash, vitamins, fiber, moisture, amino acids, minerals and antinutrients were examined using standard tests. No difference was observed between levels of these constituents in the hybrid 1507 when compared to commercial hybrids not encoding this gene, however, the non-essential amino acid, glutamic acid, was slightly above the known ranges for both the control and test lines. CLASSIFICATION: ACCEPTABLE	452748-02
Equivalency of microbial and maize expressed Cry1F protein; Characterization of test substances for biochemical and toxicological studies.	Standard techniques of protein chemistry were used to assess similarities between the bacterial and plant sources of the Cry1F protein. Additionally, insect mortality assays were performed to determine *in vitro* toxicity. An *in vitro* digestibility assay was done to determine that Cry1F was unstable under conditions simulating the gastric environment. This simulation of gastric conditions indicated that the toxin (from microbial source) was readily digested by pepsin. SDS-PAGE and Western blotting of plant and bacterial sources determined the presence of a 65 kDa protein corresponding to the trypsinized core of the c-endotoxin. Plant extracts contained 0.158 % Cry1F as determined by ELISA; control plants were negative. N-terminal sequencing of 5 aa determined that the microbial and plant expressed protein maintained this sequence intact. Glycosylation was not evident in Cry1F from either source. CLASSIFICATION: ACCEPTABLE	447149-03

2. CRY1F Event DAS-06275-8

The Agency's detailed assessment of the product characterization data for corn containing event DAS-06275-8 is found in Wozniak (2002) and Matten (2005b). The *cry*1F gene in event DAS-06275-8 codes for the identical truncated Cry1F protein as that expressed by maize plants containing the *cry*1F gene in event TC1507 (MRID 447148-01). Changes were made to the gene to alter expression in maize line 6275 plants, but these changes do not alter the amino acid sequence of the protein as compared to that expressed in maize line 1507 plants. The first 605 amino acids of the Cry1F protein in event DAS-06275-8 and event TC1507 are identical with the exception of an altered residue at position 604 (F604L). Because Cry1F proteins in the two events share an identical amino acid sequence, the protein equivalency and insect-pest spectrum data also support the Cry1F event DAS-06275-8 registration.

Specific event DAS-06275-8 product characterization data (summarized below) indicate that plant-produced Cry1F protein in maize line 6275 is biologically, biochemically, and immunologically similar to that expressed in the source bacterium *B. thuringiensis* after trypsin digest. Southern blot data of restriction enzyme digests suggests that the insert in *B.t.* Cry1F corn line 6275 occurred as a simple integration of a partial copy of the T-DNA region (truncated at the 5' end) from plasmid PHP12537. One intact copy of *bar* (plant selectable marker gene, phosphinothricin acetyltransferase,

bar) was confirmed. Southern blot analyses also revealed that tetracycline and spectinomycin resistance genes were not integrated into corn line 6275. Southern hybridization was used to assess the genetic stability of the insert in multiple generations of corn line 6275. Based on the segregation analyses, corn line 6275 exhibited stable Mendelian inheritance of the insert across the generations examined.

Study	***Result***	***MRID#***
Product Characterization Data for *Bacillus thuringiensis* var. *aizawai* moCry1F Insect Control Protein as Expressed in Maize	A synthetic, truncated *cry*1F transgene was optimized for maize expression and transformed into maize plants using *Agrobacterium tumefaciens* for plant transformation (called mo*cry1F).* The mo*cry*1F gene encodes a truncated, core insecticidal toxin that is identical in amino acid sequence to the native Cry1F protein over the first 605 amino acids with the exception of an altered residue at position 604 (F604L). The codons for the remaining C-terminal (569) amino acids of the full-length protoxin, were removed from the transgene sequence. PCR verification of the *cry1F* gene indicated that the two genes segregate as a single gene. CLASSIFICATION: ACCEPTABLE	453186-01
Quantitative ELISA Analysis of Cry1F Protein Expression Levels in Hybrid and Inbred Lines of Maize event TC6228	A direct double antibody sandwich ELISA was developed to quantify Cry1F found in lyophilized tissues of mo*cry1F* maize. Samples of leaf, pollen and grain from the two locations in Chile all produced measurable levels of Cry1F for both transformed inbred and hybrid lines when expressed on a tissue dry weight or total extractable protein basis. Leaf tissue samples indicated a higher level of Cry1F expression or accumulation as compared to pollen and grain samples. The quality control samples included in the ELISA plates yielded 73.9 to 106.2% of predicted value, which is within the realm of variation typically seen in ELISA when protein are mixed and processed along with whole tissue samples Extractions from known moCry1F corn gain samples demonstrated that a sample as small as 50 mg could be properly extracted and quantified. CLASSIFICATION: ACCEPTABLE	453186-02
Characterization of DNA Inserted into Transgenic Corn Event TC6228	The integration pattern of mo *cry1F* and *bar* genes introduced into event TC6228 plants was analyzed by Southern blotting. Control DNA spiked with PHP12537 DNA at concentrations equivalent to 1, 3 or 5 gene copies / genome was included as a positive control and a means to estimate copy number by comparing hybridization intensity. Control DNA without plasmid DNA was also included as a negative control. Data indicate that a single integration of a complete and functional transcriptional unit, representing the T-DNA of the binary plasmid PHP12537, is present in the modified corn line TC6228. Two antibiotic resistance genes, *spc* and *tet*, which are present in the region of the plasmid outside the T-DNA borders, were not transferred as determined by lack of hybridization with TC6228 DNA. CLASSIFICATION: ACCEPTABLE	452646-01
Characterization of DNA inserted into transgenic	Southern blot data from restriction enzyme digests suggest that there is a single insertion of a partial copy of the T-DNA region	460193-01

Study	***Result***	***MRID#***
corn Event TC6275	from plasmid PHP 12537 at one locus in corn line 6275. Restriction digests of corn line 6275 indicated that the *bar* gene was inserted intact and that the (mo)*cry1*F transcription unit was truncated on the 5' end up to and including the restriction enzyme site. The absence of the bacterial tetracycline and spectinomycin resistance genes and regions immediately outside of the left and right T-DNA borders was confirmed suggesting that only DNA contained within the T-DNA borders of plasmid PHP 12537 was integrated into maize line 6275. The inserted DNA was also characterized in two distinct generations of *B.t.* moCry1F maize line 6275, demonstrating the stability of the inserted DNA. CLASSIFICATION: ACCEPTABLE	
Detailed molecular characterization of the DNA inserted into transgenic corn Event TC6275	This is a second study to characterize the inserted synthetic transgene *cry*1F in *B.t.* moCry1F maize line 6275 that contains the (mo)*cry*1F and *bar* genes (see first study, MRID#460193-01). DNA extracted from corn leaf tissue was examined by Southern blot analysis to characterize the T-DNA insert containing the (mo)*cry*1F and *bar* genes in transgenic corn event TC6275 . Southern blot data from this study confirm a single insertion of the T-DNA region from PHP 12537 in event TC6275 with a T-DNA truncation or alteration of the ubiquitin promoter and intron regions Additional border fragments at the 5' end resulting from *Eco*R I digestions suggested that the endonuclease restriction site at bp 1584 in the T-DNA insert was lost during integration into the corn genome. CLASSIFICATION: ACCEPTABLE	460193-02
Nutrient composition and Cry1F and BAR protein expression levels in maize hybrid, inbred, and progenitor lines containing Event TC6275 [Expression data only.]	Cry1F and bar proteins were found in all transgenic lines, tissue types, and at almost all growth stages (none was detected in a few 6275H pollen samples and in most senescent leaf samples). On a dry weight basis, Cry1F levels ranged from a low of 0.71 ng/mg in senescent leaves (16.7 ng/mg in V9 leaves) in unsprayed transgenic lines to 44.8 ng/mg in R4 leaves; 3.7 ng/mg in pollen (unsprayed and sprayed transgenic lines); 10.4-11.0 ng/mg in stalks (unsprayed and sprayed transgenic lines); 5.82-6.26 ng/mg in forage tissue; 1.97 ng/mg in senescent roots (unsprayed transgenic line) to 6.60 ng/mg in R1 roots; and 1.08-1.14 ng/mg in grain (unsprayed and sprayed transgenic lines). On a dry weight basis, bar levels ranged from 0 ng/mg in senescent leaves to 682 ng/mg in R4 leaves (unsprayed transgenic line); 41 ng/mg in senescent roots to 373 ng/mg in R1 roots (sprayed transgenic line); 0-0.62 ng/mg in pollen (unsprayed and sprayed transgenic lines); 282-311 ng/mg in stalks (unsprayed and sprayed transgenic lines); 7-11 ng/mg in forage tissues (unsprayed and sprayed transgenic lines); and 23 ng/mg in grain (unsprayed and sprayed transgenic lines). CLASSIFICATION: ACCEPTABLE	460193-03

Study	***Result***	***MRID#***
Characterization of Cry1F protein derived from *Pseudomonas fluorescens* and transgenic corn	This study characterized the Cry1F insecticidal protein derived from transgenic corn plant event TC6275 and from the *Pseudomonas fluorescens* bacterium and compared their physical/chemical properties. The Cry1F protein produced in transgenic plants was sensitive to protease cleavage at its N–terminal end. The resulting toxic core protein had a molecular weight of 65 kDa. This core protein was equivalent to the trypsin-truncated microbe-derived Cry1F protein. The Cry1F protein from both sources showed no measurable glycosylation. Tryptic peptide mass fingerprints using MALDI TOF MS, N–terminal sequencing using Edman degradation, and insecticidal all provide supporting evidence that microbially-produced (i.e., *P. fluorescens*) and transgenic maize-produced Cry1F proteins are equivalent molecules. CLASSIFICATION: ACCEPTABLE	460193-04
Characterization of phosphinothricin acetyltransferase (BAR) derived from recombinant *E. coli* and transgenic corn	The study demonstrated the molecular identity of the BAR protein produced by *E. coli* strain MR1513 and that produced in transgenic corn event 6275. Both sources produced a BAR protein with the expected molecular weight of ~ 21 kDa, and both proteins were immunoreactive to a specific polyclonal antibody against the BAR protein. The BAR protein produced by both the microbe and corn sources lacked detectable post-translational glycosylation. MALDI-TOF MS analysis of tryptic digests of the BAR proteins and N–terminal sequence analysis done by Edman degradation provided additional evidence that the BAR proteins produced by *E. coli* and transgenic corn event 6275 are biochemically equivalent molecules. CLASSIFICATION: ACCEPTABLE	460630-01
Development and characterization of enzyme-linked immunosorbent assay (ELISA) for the detection of Cry1F protein	This study demonstrates the high quantitative performance of the ELISA assay for the detection of the Cry1F truncated protein. The assay had a reproducible sensitivity of 0.5 ng/mL with an approximate 40-fold assay range of 0.4 to 17 ng/mL truncated Cry1F). The coefficient of variation (%CV) of the absorbance measurement within the curve is less than 10%. The cross-reactivity profile indicated little to no cross-reactivity or interference from a standard panel of agriculturally relevant recombinant proteins. The Cry1F assay kit was projected to be stable for approximately 1 year at 4ºC. CLASSIFICATION: ACCEPTABLE	456856-01
Independent Laboratory Validation of Method GRM 02.13, Determination of Cry1F delta-Endotoxin Protein in Corn Grain by Enzyme Linked-Immunosorbent Assay	An independent laboratory, EPL Bio-Analytical Services, validated Dow AgroSciences LLC analytical method GRM°02.13 "Determination of Cry1F Insecticidal Crystal Protein in Corn Grain by Enzyme Linked Immunosorbant Assay" for accuracy, precision, and sensitivity. The LOQ of the method was confirmed as 0.75 μg/g (or 0.5 ng/mL) for corn grain extracts spiked with Cry1F. Average recoveries from samples spiked with Cry1F protein averaged 99 and 90 percent at the 0.075 and 0.15 μg/g spike levels, respectively. The relative standard deviation (RSD) of replicate recovery	456856-02

Study	*Result*	*MRID#*
	measurement did not exceed 20 percent at or above the LOQ and interferences were negligible (<20% of the response of the Cry1F protein at the LOQ of 0.075 ⌋g/g). The results of this assay validation indicate that the ELISA based assay is suitable for the analysis of Cry1F as found in corn grain. CLASSIFICATION: SUPPLEMENTAL; upgradeable with additional information regarding whether there is interference in detection of the Cry1F protein in transgenic Cry1F corn grain	

B. HUMAN HEALTH ASSESSMENT

The detailed Agency human health assessment of 1507 corn is found in EPA (2001 a and b). The 6275 corn is found in Wozniak (2002) and Matten (2005a and b). A summary of the key findings is provided below.

1. Bridging from poCry1F to moCry1F

Because the Cry1F proteins from event DAS-06275-8 and event TC1507 share the same amino acid sequence, the toxicity of the Cry1F protein expressed in 1507 and 6275 corn plants is expected to be similar. The toxicity and allergenicity data submitted in support of the event TC1507 registration are also adequate to support the registration of event DAS-06275-8 corn.

2. Mammalian Toxicity and Allergenicity Assessment

Based upon the human health data provided, there is a minimal risk of toxic and/or allergenic effects to humans or animals due to exposure to the Cry1F protein. Based on review of the data, there is a reasonable certainty of no harm to humans and animals posed by the aggregate exposure to residues of this protein.

Dow AgroSciences submitted acceptable waiver requests for certain human health safety data for Guidelines 152-31-36 (Matten, 2005a). 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 several fold greater than the maximum possible exposure levels that are reasonably anticipated in the crops. This is similar to the Agency position regarding maximum hazard toxicity testing and the requirement of residue data for the microbial *Bacillus thuringiensis* products from which this plant-incorporated protectant was derived. [See 40 CFR Sec. 158.740(b)(2)(i).] For microbial products, further toxicity testing and residue data are triggered by significant adverse acute effects in studies such as the mouse oral toxicity study, to verify the observed adverse 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 at >5050 mg/kg body weight (0.576

g/kg of Cry1F in a solution containing 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 volume totaling 33.7 mL / kg body weight. Outward clinical signs were observed and body weights 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 LD50 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 (Toxicity category III based on dose given with no observable effect). At this dose, an LD_{50} could not be calculated because there was no mortality throughout the 14 day study. Cry1F DAS-06275-8 maize seeds contain approximately 0.0011 mg of Cry1F/g of maize kernel tissue. Cry1F TC1507 maize seeds contain approximately 0.0017 to 0.0034 mg of Cry1F/gram of maize 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 Cry1F plant-incorporated protectant, even at relatively high dose levels tested, 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. 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. There was no identity of 35% or greater over 80 amino acid residues between the Cry1F and any known allergens. A match was identified between the Cry1F protein of Herculex I (TC1507) corn and the Der p7 protein, an allergenic protein of the dust mite, *D. pteronyssinus* using a 6 contiguous amino acid bioinformatics search. Although it is generally agreed that sequence identity over 6 amino acids but not 8 is not an indication of a possible cross-reactive allergic response, a cross-reactivity test was conducted. No cross-reactivity between Cry1F protein in 1507 maize and dust mite Der p7 protein was observed when tested with human sera positive for Der p7-IgE. Dust mite allergic individuals would not be expected to experience an allergic reaction from ingesting Cry1F. In addition, Cry1 proteins have not been implicated in toxic and/or allergenic reactions in humans or animals. Based on the weight-of-the evidence, the potential for the Cry1F protein to be a food allergen is minimal. Given the lack of findings suggesting potential allergenicity and the absence of adverse effects in the acute oral toxicity test, there is no finding to suggest an adverse immune response nor a potential for mammalian toxicity from the Cry1F protein.

3. 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-incorporated protectant chemical residue, and exposure from non-occupational sources. Exposure via the skin or inhalation is minimal, but there is no evidence of mammalian toxicity through these routes of exposure. 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-incorporated protectants 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.

4. 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-incorporated protectants, we conclude that there are no cumulative effects for the Cry1F protein.

5. 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 acceptable 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 (EPA, 2001a and b; Matten, 2005b). Microbially produced protein was chosen in order to facilitate

obtaining 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 maximum hazard dose toxicity testing and the requirement of residue data for the microbial *Bacillus thuringiensis* products from which this plant-incorporated protectant was derived. [See 40 CFR Sec. 158.740(b)(2)(i).] For microbial products, further toxicity testing and residue data are triggered by significant adverse acute effects in studies such as the mouse oral toxicity study to verify the observed adverse 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-incorporated protectant 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 several fold greater than the possible maximum exposure levels anticipated in the crop.

Residues of nucleic acids that are part of a plant-incorporated protectant are exempt from the requirement of a tolerance (40 CFR 174.475).

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-incorporated protectants.

6. 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 incorporated protectants at this time.

b) Analytical Method(s)

Because the two Cry1F proteins share an identical amino acid sequence, the same analytical method is used for both 1507 corn and 6275 corn. The method for extraction and direct ELISA analysis of Cry1F in corn grain has been found to be acceptable by the Agency (MRID 45685601). The independent laboratory validation of the ELISA method has been found to be supplemental, but is upgradeable with additional information regarding whether there is interference in detection of the Cry1F protein in transgenic Cry1F corn grain (MRID 45685602).

c) Codex Maximum Residue Level

No Codex maximum residue level exists for the plant-incorporated protectants *Bacillus thuringiensis* Cry1F protein and the genetic material necessary for its production in corn.

7. Tolerance Exemptions

An existing tolerance exemption, CFR 40 Section 180.1151, exists for phosphinothricin acetyltransferase and the genetic material necessary for its production in all plants. The BAR (PAT) protein expressed in Mycogen Brand Cry1F Event DAS-06275-8 corn is covered by the existing tolerance exemption, CFR 40 Section 180.1151. The data submitted and reviewed for event DAS-06275-8 support the tolerance exemption for *Bacillus thuringiensis* Cry1F protein and the genetic material for its production in corn, 40 CFR 180.1271.

8. Supporting Data

Study	Result	MRID #
Acute oral toxicity study in mice: Cry1F *Bacillus thuringiensis* var. *aizawai* delta-endotoxin.	Dosing of ten albino mice with bacterial cell protein containing the d-endotoxin of *Bacillus thuringiensis* var. *aizawai* at > 5050 mg/kg (0.576 g/kg of Cry1F) body weight resulted in no mortality and no observed gross abnormalities. All animals appeared normal during the study and all except one gained weight throughout the study. CLASSIFICATION: ACCEPTABLE. Toxicity category III based on dose given with no observable effect.	446911-01
Supplement to MRID 446911-01: Supplemental Data for Acute Oral Toxicity Study in Mice: Cry1F *Bacillus thuringiensis* var. *aizawai* delta-endotoxin	This submission represents a clarification of test substance as presented in a previous submission and review. The acute oral toxicity study dosed mice at > 5050 mg microbial protein / kg body weight. 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. The truncated form of the protein represents amino acids 28-612 of the Cry1F toxin sequence, whereas the plant-expressed form of Cry1F contains amino acids 1-605. The truncated form used in the oral toxicity study adequately represents that toxin to be found in the plant expression system. CLASSIFICATION: ACCEPTABLE	450201-18
Comparison of amino acid sequence similarity of Cry1F and PAT proteins to known allergen proteins	As part of the assessment of allergenicity potential of Cry1F, a bioinformatics analysis was conducted to compare the amino acid sequence of Cry1F and PAT with sequences in a database of food, dermal, and respiratory allergens. Homology (≥ 35% over any 80 amino acid overlap) was not detected using the FASTA algorithm, and no contiguous amino acid sequence matches were identified using an eight amino acid search against known allergens. For both proteins of interest, the lack of any significant amino acid homology indicates that the potential for an immunological response developing into a food allergy from consumption of these proteins is low. CLASSIFICATION: ACCEPTABLE	449717-01
Equivalency of microbial and maize expressed Cry1F protein; Characterization of test substances for biochemical and toxicological studies.	Standard techniques of protein chemistry were used to assess similarities between the bacterial and plant sources of the Cry1F protein. Additionally, insect mortality assays were performed to determine *in vitro* toxicity. An *in vitro* digestibility assay was done to determine that Cry1F was unstable under conditions simulating the gastric environment. This simulation of gastric conditions indicated that the toxin (from microbial source) was readily digested by pepsin. SDS-PAGE and Western blotting of plant and bacterial sources determined the presence of a 65 kDa protein corresponding to the trypsinized core of the delta-endotoxin. Plant extracts contained 0.158 % Cry1F as determined by ELISA; control plants were negative. N-terminal sequencing of 5 aa determined that the microbial and plant expressed protein maintained this sequence intact. Glycosylation was not evident in Cry1F from either source.	447149-03

	CLASSIFICATION: ACCEPTABLE	
Thermolability of Cry1F (truncated) Delta-Endotoxin	The Cry1F test substance was prepared in 10 mM potassium phosphate buffer (pH 7.5) and placed into a water bath at either 60, 75 or 90 °C for 30 minutes, or into the refrigerator at 4 °C. Application of treated Cry1F to the surface of an insect diet and measurement of growth inhibition of neonate tobacco budworm larvae, indicated that the Cry1F protein was labile to heat at and above 75 °C. CLASSIFICATION: ACCEPTABLE	452748-01
Comparison of the amino acid sequence of phosphinothricin acetyltransferase protein to known protein allergens	The results of the bioinformatics comparison indicated that there were no alignments of eight or more contiguous identical amino acids between the BAR protein and any of the proteins in the allergen database. Also, there was no identity of 35% or greater over 80 amino acid residues between the BAR sequence and any of the sequences in the two allergen databases. There was, therefore, no immunologically significant sequence identity between the amino acid sequence of the BAR protein and known protein allergens. CLASSIFICATION: ACCEPTABLE	460193-09
Bialophos resistance protein: acute oral toxicity study in CD-1 mice	The oral LD_{50} for males, females, and combined was greater than 5000 mg microbial protein/kg (or >3250 mg Bialaphos Resistance Protein (BAR)/kg body weight). This places Bialaphos Resistance Protein (65% a.i.) in TOXICITY CATEGORY IV. CLASSIFICATION: ACCEPTABLE	460630-02
In vitro simulated gastric fluid digestibility study of recombinant phosphinothricin acetyltransferase	The purpose of this study was to evaluate the behavior of the microbe-derived BAR protein in simulated gastric fluid (SGF). Based on SDS-PAGE and Western blots, the time at which >98% of the BAR protein was degraded was <0.5 minutes (DT_{98} < 0.5 minutes). Therefore, BAR protein is readily digested by pepsin under simulated gastric conditions (pH=1.2). CLASSIFICATION: ACCEPTABLE JkIj;kIjkI;j;lkjlkjkI;	460630-03
Lack of cross-reactivity between Cry1F protein in Herculex I maize and dust mite Der p7 protein with human sera positive for Der p7-IgE	Using a six contiguous amino acid bioinformatics analytical search, a match was identified between the Cry1F protein of Herculex I (poCry1F) corn and the Der p7 protein, an allergenic protein of the dust mite, *D. pteronyssinus*. This study examined cross-reactivity between Cry1F protein in Herculex I maize and dust mite Der p7 protein with human sera positive for Der p7-IgE. There was no cross-reactivity; therefore, dust mite allergic individuals would not be expected to experience an allergic reaction from ingesting Cry1F. CLASSIFICATION: ACCEPTABLE	464440-01

C. ENVIRONMENTAL ASSESSMENT

1. Ecological Effects Hazard Assessment

This environment hazard assessment includes outcrossing and potential for weeds to develop if pollen from 1507 and/or 6275 corn was to fertilize other plants, horizontal gene transfer, expression of Cry1F protein in 1507 and 6275 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. In 2001, the Agency conducted an environmental effects assessment of Cry1F, the insecticidal protein as expressed in maize line 1507 (EPA, 2001b). In 2005, the Agency conducted an environmental effects assessment of Cry1F, the insecticidal protein as expressed in maize line 6275 (Hill, 2005). The assessment of maize line 6275 is based primarily on the bridging of data submitted to the Agency for 1507 corn (EPA Reg. No. 029964-3; EPA, 2001b).

2. Bridging of Event TC1507 Data to Include Event DAS-06275-8

The *cry*1F gene in event DAS-06275-8 encodes for the identical truncated Cry1F protein as expressed by maize plants containing the *cry*1F gene in event TC1507 (MRID No. 447148-01). Changes were made to the gene to modify expression in 6275 maize plants but these changes did not alter the amino acid sequence of the protein as compared to that expressed in 1507 maize plants. Therefore, the specificity (i.e., toxicity toward target pests) of the Cry1F protein expressed in 6275 and 1507 plants should be similar. Testing with bacterially prepared Cry1F protein at levels greatly exceeding those found in maize optimized plants resulted in no effect with several beneficial species including the monarch butterfly (**Tables 1 & 2**). Although, the expression data reveal that 6275 plants express somewhat lower concentrations of Cry1F protein in pollen and grain and higher levels of Cry1F protein in stalks, leaves, and roots than the 1507 plants, these expression differences are not expected to significantly change the exposure to lepidopteran non-target organisms **(Table 3)**. Field monitoring for effects of 1507 corn on non-target insects confirmed the absence of adverse effects to non-target organisms (MRID No. 450201-13; **Table 1**). Data illustrating the soil degradation of Cry1F protein in event TC1507 were submitted to the EPA in support of the registration of Herculex I (MRID No. 450201-05). These data support the registration of 6275 corn because the two proteins share an identical amino acid sequence. EPA's assessment of the 1507 studies submitted in support of the Herculex I registration (EPA Reg. No. 029964-3; EPA, 2001b, **Tables 1 & 2**) are applicable to the proposed 6275 corn registration for reasons outlined above. These studies will be briefly summarized below in both a tabular format (**Table 2**) and a more descriptive format.

Table 1. PoCry1F studies submitted in support of Herculex I registration (EPA Reg. No. 029964-3) and bridged to MoCry1F.

Toxicity to Non-Target Organisms	
Study Title	**MRID No**
Cry1F Bt var. aizawai delta-endotoxin: Acute oral toxicity study in mice	446911-01
Chronic exposure of *Folsomia candida* to bacterially expressed Cry1F protein	450201-07
Waiver request: fish toxicity test with transgenic maize (corn) containing *Bacillus thuringiensis* var. aizawai (Bt) Cry1F delta-endotoxin	450442-01
Toxicity of the Cry1F protein to neonate larvae of the monarch butterfly (*Danaus plexippus* (Linnaeus))	451311-02
Cry1F *Bacillus thuringiensis* var. aizawai delta-endotoxin: a dietary toxicity study with the ladybird beetle	450201-10
Cry1F *Bacillus thuringiensis* var. aizawai delta-endotoxin: a dietary toxicity study with green lacewing larvae	450201-09
Cry1F *Bacillus thuringiensis* var. aizawai delta-endotoxin: a dietary toxicity study with parasitic hymenoptera	450201-11
Evaluation of the dietary effect(s) on honeybee development using bacterially expressed *Bt* Cry1F delta-endotoxin and pollen from maize expressing *Bt* Cry1F delta-endotoxin	450201-06
Bt Cry1F delta-endotoxin: a 48-hour acute toxicity test with the cladoceran (*Daphnia magna*) using bacterially-expressed Bt Cry1F delta-endotoxin, and pollen from maize expressing Bt Cry1F delta-endotoxin	450201-08
Cry1F *Bacillus thuringiensis* var. aizawai delta-endotoxin: an acute toxicity study with the earthworm in an artificial substrate	452021-06
Transgenic corn expressing *Bacillus thuringiensis* var. aizawai (Bt) Cry1F delta-endotoxin: a dietary toxicity study with the Northern bobwhite	450201-12
Field survey of beneficial arthropods associated with *Bacillus thuringiensis* Cry1F maize	450201-13
Non-target exposure and risk assessment for environmental dispersal of Cry1F maize pollen	450415-02
Degradation in Soil	
Study Title	**MRID No.**
Environmental fate of Cry1F Protein Incorporated into Soil	450201-05

Table 2. Tabular results of non-target wildlife testing.

Guideline No.	Study	Results	MRID No.
155-18	Environmental Fate of Cry1F Protein Incorporated Into Soil	DT_{50} of 3.13 days. Cry1F will degrade in the soil within 28 days (the duration of this test). The study was determined to be **Supplemental** and it was recommended that the soil degradation study be carried out for a longer period of time to determine the duration and the amount of residual Cry 1F protein in agricultural soil.	450201-05
USEPA OPPTS 885.4150	Wild Mammal Testing, Tier I	Mammalian wildlife exposure to moCry1F protein is considered likely; however, the mammalian toxicology data submitted for the Human Health Assessment for poCry1F indicates that there was no significant toxicity to rodents from acute oral testing at the maximum hazard dose. Based on the bridging data in combination with the poCry1F rodent study, no hazard to mammalian wildlife is anticipated from moCry1F. **Acceptable.**	446911-01
71-2, 154-7	Dietary Toxicity Study with the Northern Bobwhite (*Colinus virginianus*)	The dietary LC_{50} for corn grain (meal) expressing *Bt* var. aizawai protein in corn grain when fed to juvenile northern bobwhite quail (*Colinus virginianus*) for five days was determined to be greater than 100,000 ppm (10% of corn meal). The NOEC was 100,000 ppm and there were no treatment-related mortality or behavioral changes observed in comparison to the control replicates. These data were determined to be insufficient to make a hazard assessment from repeated exposure(s) to higher doses of Bt corn and a six week study with 60 to 70% corn in the diet was deemed necessary to assess hazards from chronic exposure of wild and domesticated fowl. Therefore, the study was determined to be supplemental. However, the additional study was submitted (see below) and the study is now upgraded to **Acceptable**.	450201-12
Non-guideline	Nutritional Equivalency of Bt Cry1F Maize-Poultry (Cobb x Cobb) Feeding Study	In a six week study where commercial broiler chickens were fed a diet containing 54.21%-57.03% *Bt* Cry1F line 1507 and control diets there was no statistically significant difference found in mortality, mean body weight, mean daily weight gain, or mean food conversion. **Acceptable.**	456220-01
USEPA OPPTS 885-4380	Honey Bee Larva Testing, Tier I	The data show that at 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*). There was no treatment mortality or behavior change observed between the dosed and control replicates. LC_{50} > 64 ng Cry1F in 2 mg pollen /larva and 640 ng Cry1F protein /larva. Based on the bridging data in combination with this study, Cry1F protein as expressed in corn pollen should have no detectable adverse effects on honey bee larvae or their development into healthy adults. **Acceptable.**	450415-03, 453078-05 (supplement)
885.4340	Non-target Insect Testing, Tier I with Green Lacewing Larvae (*Chrysoperla carnea*)	Green lacewing larvae fed a concentration of Bt poCry1F protein at15x the expected rate found in corn pollen (pollen expressing 32 ng Cry1F/mg pollen) resulted in no mortality or signs of toxicity or abnormal behavior over a 13 day period (>20% control mortality period). The LC_{50} and NOEC was determined to be >15x the concentration of poCry1F found in pollen and the was determined to be > 480 ppm a.i (the test concentration). Mortality and pupation rate were comparable between the treatment and control group. Based on the bridging data in combination with the poCry1F green lacewing study moCry1F should have no detectable adverse effects on *Chrysoperla carnea* in the field. **Acceptable.**	450201-09, 453078-01 (supplement)
885.4340	Non-target Insect Testing, Tier I with the Ladybird Beetle (*Hippodamia convergens*)	Adult lady beetles fed a concentration of Bt Cry1F protein at at 15x the expected rate found in corn pollen (pollen expressing 32 ng Cry1F/mg pollen) resulted in no mortality or signs of toxicity over a 29 day period. Therefore, the NOEC and the LC_{50} were determined to be >15x the	450201-10, 453078-02 (supplement)

Table 2. Tabular results of non-target wildlife testing.

Guideline No.	Study	Results	MRID No.
		concentration of Cry1F found in pollen determined to be > 480 ppm a.i (the test concentration). 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. **Acceptable**.	
885.4340	Non-target Insect Testing, Tier I with the Parasitic Hymenoptera (*Nasonia vitripennis*)	Parasitic Hymenoptera fed a concentration of Bt Cry1F protein 10x the expected rate found in corn pollen (expressing 32 ng Cry1F/mg pollen) showed no mortality or signs of toxicity or abnormal appearance or behavior of surviving wasps in the treatment or control group over a 12 day period. The test was terminated after 12 days because 20% mortality was reached in the negative control. The NOEC and the LC_{50} were determined to be > 320 ppm a.i (10x field rate when calculated for pollen expressing 32 ng Cry1F/mg pollen). No hazard to parasitic Hymenoptera at field use rates is expected from the cultivation of Cry1F containing corn. **Acceptable**.	450201-11, 453078-03 (supplement)
885.4340	Non-target Insect Testing, Tier I with Neonate Larvae of the Monarch Butterfly (*Danaus plexippus*)	First instar Monarch larvae fed a 10,000 ng/mL diet (the highest rate tested) showed no mortality after seven days of feeding. There was some growth inhibition at 10,000 ng/mL diet. LC_{50} > 10,000 ng/mL and NOEC <10,000 ng/mL. Since doses equivalent to 10,000 ng/mL diet are not likely to occur in nature, it was concluded that Cry1F protein will not pose a risk to monarchs. **Acceptable**.	451311-02
885-4340	Collembola Testing, Tier I (*Folsomia candida*)	Collembola (*Folsomia candida*) were fed three treatment levels (12.5, 3.1, 0.63 mg/kg) of Cry1F protein every two to three days for 28 days representing 79x, 388x, and 1560x that which would be encountered in the field with no observable treatment mortality or behavior change. Results of the study indicate that levels of Cry1F that might occur in the field are not expected to adversely effect the soil invertebrate Collembola species. LC50 and NOEL >12.5 mg Cry1F/kg soil. **Acceptable**.	450201-07
OECD Guideline 207	Acute Toxicity Study with the Earthworm (*Eisenia fetida*) in an Artificial Soil Substrate	Earthworms, *Eisenia fetida*, fed 2.26 mg poCry1F/kg dry soil, representing up to 100X the estimated concentration present in the top six inches of an acre of soil following the incorporation of 25,000 senescent corn plants did not have adverse effects. LC50 and NOEL >2.26 mg Cry1F/kg dry soil. Based on the results of this study, it is not likely that Cry1F transgenic corn plantings will have adverse effects on earthworms. **Acceptable**	450201-06, 453078-04 (supplement)
154-35	Non-target Insect Field Survey	Sticky traps were set out weekly for six weeks. In addition, ten plants in the center row were visually evaluated for beneficial arthropods weekly for six weeks. Beneficial insects counted 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), ichneumonids and braconids (parasitic wasps), damselflies and dragonflies (Odonata), and spiders (Arachnida). Data included counts of adult and larval lady beetles and lacewings when appreciable numbers were collected. Results from the study indicated that the transgenic corn lines TC1507 and 1360 did not adversely affect the number of beneficial arthropods in the field. In general, line TC1507 showed larger numbers of beneficial insects. The field census study adequately addressed potential concerns for Cry1F protein expressed in corn to non-target insect populations. However, the Agency recommended that the monitoring continue into the first few years of commercial use of Cry1F corn crops in	450201-13

Table 2. Tabular results of non-target wildlife testing.

Guideline No.	Study	Results	MRID No.
		order to confirm the single season "no effects" findings and to gather long-range non-target insect effects and abundance data. **Acceptable**.	
N/A	Non-target Exposure and Risk Assessment for Environmental Dispersal of Cry1F Maize Pollen. (A probabilistic risk assessment)	This study was conducted with poCry1F to consider the exposure of non-target species including endangered Lepidoptera species to field corn pollen expressing the Cry1F delta endotoxin by evaluating pollen dissemination. The Cry1F concentration found in pollen occurring on milkweeds near the edge of Bt corn fields was predicted. Distance of pollen dispersal, levels of Cry1F expression in pollen, milkweed distribution and biomass from the edge of the field, pollen grain physical properties, and spatial-temporal availability of Cry1F to monarch larvae was determined. According to a probability-log plot demonstrating lepidopteran species susceptibility to Cry1F, 99% of lepidopteran species exhibit an LC_{50} of ≥ 0.06 $\mu g\ g^{-1}$ which is 290-fold lower than the geometric mean LC_{50} (12.4 $\mu g\ g^{-1}$) and lower than the most sensitive lepidopteran species. The toxicity threshold, or no effect level for monarch neonates, for the Tier 1 risk assessment was determined to be 10 $\mu g\ g^{-1}$ diet. When fed up to 10 $\mu g\ g^{-1}$ Cry1F microbial toxin in diet, neonate monarch larvae were not affected. The toxicity threshold, or no effect level for monarch neonates, for the Tier 1 risk assessment was determined to be 10 $\mu g\ g^{-1}$. The log-probability plot of the Bt LC_{50} for lepidopteran species shows that the EEC does not exceed the LC_{50} for 98% of the intergenera population beyond 1 m from the field edge. The LC_{50} is not exceeded for 90% of the population 0.2 m from the edge. For monarch larvae, the no effect level (10 $\mu g\ g^{-1}$) occurs near the 50th percentile intergenera LC_{50}. Since there is a rapid fall-off in exposure with distance, there is limited potential for non-target effects beyond the immediate field border. In addition, the estimated risk quotients (ratio of exposure to effect) demonstrate a lack of concern for monarchs (or other lepidopteran species) beyond 1 m from the field edge. The RQ in the corn field was 0.096. Finally, pollen from moCry1F plants express less Cry1F protein than poCry1F plants, further reducing non-target exposure. **Acceptable.**	450415-02
72-2, 154-9	A 48-Hr Static Renewal Acute Toxicity Test with the Cladoceran (*Daphnia magna*)	There were no overt signs of toxicity to daphnids (*Daphnia magna*) exposed to 100 mg *Bt*-pollen/L - (maize pollen containing the *Bt* Cry1F delta-endotoxin). The 48-hr EC50 was > 100 mg a.i./L. The NOEC was >100 mg a.i./L. These data show that there will be no adverse effects on daphnia from incidental field exposure to transgenic corn pollen containing Cry1F. **Acceptable.**	450201-08
USEPA OPPTS 885-4200	Freshwater Fish Testing, Tier I	The Agency previously waived static renewal toxicity tests for freshwater fish due to the lack of substantial exposure to poCry1F protein in runoff and corn pollen. However, the registrant submitted a study in support of the potential moCry1F registration. Juvenile rainbow trout (*Onchorhynchus mykiss*) were fed a standard fish diet containing 100 mg Cry1F ICP a.i./kg of diet for eight days with no mortality or sublethal effects. The LD_{50} was determined to be greater than 100 mg a.i./kg of diet. The actual concentration of the test material in the diet was not determined and therefore this study is **Supplemental**.	450442-01 460193-06
850.1075	Fish Acute Toxicity Test, Freshwater and Marine	Study was not required for this product because of very low or no potential for exposure.	None assigned

3. Outcrossing and Weediness

The EPA has reviewed the potential for gene capture and expression of Cry1F protein by wild or weedy relatives of corn in the United States, its possessions or territories and has found that there is no significant risk in the United States, its possessions or territories (EPA 2001b). Domesticated corn does not have a reasonable possibility of passing its traits to wild maize species. Feral species related to corn (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. 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* spurred the EPA to take a closer look at this topic (EPA 2001a and b).

Upon review, the EPA concluded that the potential for pollen-directed gene flow from maize to *Tripsacum* species was extremely remote, as evidenced by the difficulty with which *Tripsacum dactyloides* x *Zea mays* hybrids are produced in structured breeding programs. Furthermore, 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 was lacking in most or all of the maize chromosomal complement in subsequent generations. 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, the EPA found that any introgression of genes into wild teosinte from *Zea mays* was not considered to be a significant agricultural or environmental risk. Furthermore, the Agency stated that the growth habits of teosintes are such that the potential for serious weedy propagation and development is not biologically plausible in the United States.

4. Ecological Exposure

a. Comparative Expression of Cry1F Protein in Event DAS-06275-8 and Event TC1507 in Corn Tissues

There is significantly increased Cry1F protein expression in corn line 6275 stalks, whole plants, and forage sampled during the flowering (R1) stage in comparison to corn line 1507 (**Table 3**). Conversely, there is significantly decreased Cry1F protein expression in pollen and grain in line 6275 in comparison to line 1507 (**Table 2**). The expression data reveal that 6275 plants express somewhat lower concentrations of Cry1F protein in pollen and grain and higher levels of Cry1F protein in stalks, leaves, and roots than the 1507 plants, these expression differences are not expected to significantly change the exposure to lepidopteran non-target organisms **(Table 3)**. The increased expression of Cry1F protein in stalks in event DAS-06275-8 hybrids could potentially decrease the risk of resistance development by borers whilst the decreased expression in pollen and grain should decrease the non-target exposure to Cry1F expressed in corn (Zabik *et. al* 2003).

Table 3. Comparative expression of Cry1F protein in Event DAS-06275-8 and Event TC1507 corn tissues (Table recreated from Zabik *et al.* 2003).

Tissue	Growth Stage	Mean	Standard Deviation	Min/Max Range	Mean	Standard Deviation	Min/Max Range
		DAS-06275-8			TC1507		
		(ng/mg Tissue Dry Weight)					
Leaf	**V9**[b] **R1** **R4** **Senescence**	17.3 28.5 44.8 0.71	3.41 5.38 16.8 1.14	10.7-23.8 16.5-36.7 35.8-109.2 0-3.0.9	12.1	6.2	0-24
Root	**V9**[b] **R1** **R4** **Senescence**	6.14 6.60 5.99 1.97	1.87 1.98 1.89 2.03	4.53-8.14 3.14-10.9 2.35-9.26 0.29-6.91			
Whole Plant	**V9** **R1** **Senescence**	6.22 7.16 2.47	1.16 1.45 0.41	4.98-7.87 5.32-9.57 1.95-3.07	5.2 3.6 1.6	1.9 1.1 0.6	2.6-6.8 2.5-4.7 0.9-2.4
Pollen	**R1**	3.67	0.34	3.09-4.60	21.9	2.9	16.4-27.2
Stalk	**R1**	11.0	2.67	6.77-16.4	5.8	1.7	3.3-10.3
Forage	**R4**	6.26	1.09	5.05-7.77	1.7	1.1	0-3.2
Grain	**Maturity**	1.14	0.27	0.62-1.68	2.2	0.8	0-4

[b] Recalculated Results

b. Degradation and Estimated Environmental Concentration

Degradation of Cry1F in the soil was demonstrated in the study submitted in support of the event TC1507 registration (MRID No. 450201-05). 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. Although the EPA stated that there is no evidence to indicate that prolonged exposure to trace amounts of Cry protein in the soil affects non-target organisms, the Agency felt that the submitted data did not sufficiently address the issue of residual Cry protein accumulation in the soil. The Agency recommended that the soil degradation study be carried out for a longer period of time to determine the duration and the amount of residual Cry 1F protein in agricultural soil (see EPA 2001b for more details). The Agricultural Biotechnology Stewardship Technical Committee (ABSTC) submitted a research protocol for a Bt

Cry protein soil accumulation study on March 15, 2002. The EPA found the proposed protocol acceptable with the additional requirement of an ELISA (letter from USEPA to ABSTC 05/08/02). The ABSTC submitted a response to the Agency's letter including a revised protocol on June 5, 2002 and the EPA responded that the revised protocol was acceptable provided that the detailed protocol of the insect bioassay is submitted and found acceptable. The Agency is awaiting this protocol and the final report which is due March 15, 2008.

The amount 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) for poCry1F protein was calculated to be 23 micrograms /kg dry soil (15 cm deep). Whole 6275 plants at the R1 stage contain 7.16 ng of Cry1F/mg of tissue (dry weight) whereas 1507 plants at the R1 stage contain 3.6 ng of Cry1F/mg of tissue (or 50% of the amount of Cry1F that moCry1F plants contain). Therefore, we can roughly approximate that the EEC for 6275 at the R1 stage would be 34.5 micrograms/kg dry soil (15 cm deep).

c. Effects on Soil Microbial Flora

Limited published data indicate that Cry proteins do not have any measurable detrimental effect on microbial populations in the soil, even at levels much higher than expected from Cry1F Bt corn cultivation.

d. Horizontal Transfer of Transgenes 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 *B. 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, antibiotic resistance genes and 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.

5. Non-Target Wildlife Toxicity Testing and Hazard Assessment

a. Hazard Characterization for Terrestrial Wildlife

i. Mammalian Wildlife Hazard Assessment

Wild Mammal Testing, Tier I USEPA OPPTS 885.4150 [MRID No. 446911-01]

Mammalian wildlife exposure to moCry1F protein is considered likely; however, the mammalian toxicology information gathered to date for poCry1F does not demonstrate a hazard to wild or domesticated mammals. Dosing of ten albino mice with bacterial cell protein containing the d-endotoxin of *Bt* var. aizawai at > 5050 mg/kg (0.576 g/kg of Cry1F) body weight resulted in no mortality and no observed gross abnormalities. All animals appeared normal during the study and all except one gained weight throughout the study. The data submitted to the EPA in support of the registration of Event TC1507 for the Human Health Assessment combined with the bridging data summarized above, indicate that there is no significant toxicity to rodents from acute oral testing of Cry1F protein at the maximum hazard dose and therefore no hazard to mammalian wildlife is anticipated from the Cry1F protein from event DAS-06275-8.

ii. Avian Hazard Assessment [Avian Oral, Tier I USEPA OPPTS 885.4050 [MRID Nos. 450201-12 & 456220-01 (supplement)]]

The dietary LC_{50} for corn grain (meal) expressing *Bt* var. aizawai protein in corn grain when fed to juvenile northern bobwhite quail (*Colinus virginianus*) for five days was determined to be greater than 100,000 ppm (10% of corn meal). The no-observed-effect concentration (NOEC) was 100,000 ppm and there were no treatment-related mortality or behavioral changes observed in comparison to the control replicates. The study was determined to be supplemental and that a six week study with 60-70% Bt corn in the diet was necessary to determine hazards from chronic exposure of wild and domesticated fowl (EPA 2001b).

The registrant submitted a six week study as required by the EPA (MRID No. 456220-01). Two hundred forty-five male broiler chickens (Cobb x Cobb) were fed diets containing commercial corn-soy type ration with either reference yellow dent corn, Bt Cry1F maize 1507 hybrid, or a non-transgenic control hybrid corn (five replications each) for six weeks. The broilers were fed a starter ration (54.21% corn) for the first 20 days and then a grower ration (57.03% corn) for days 21 through 42. There were no statistically significant differences in mean percent mortality, mean body weight, mean daily weight gain, or mean feed conversion among any of the treatments.

Furthermore, grain from corn line 6275 contains less Cry1F protein (1.14 ng/mg) as compared to grain from line 1507 which contains 2.2 ng/mg of Cry1F protein (dry weight tissue; Zabik *et. al* 2003). The six week broiler study in conjunction with the initial bobwhite quail study submitted in 2001 are sufficient to demonstrate that there should be no discernible detrimental effects to wild or domesticated fowl from the proposed uses of Cry1F producing corn including event DAS-06275-8.

iii. Terrestrial Invertebrate Testing

All of the insect studies submitted in support of the registration of Cry1F used maximum hazard dose concentrations of Cry1F event TC1507 corn pollen. As shown above (**Table 3**), there is higher Cry1F protein expression in TC1507 corn pollen (21.9 ng/mg tissue) as compared to 3.67 ng/mg tissue in DAS-06275-8 pollen (dry weight tissue; Zabik *et. al* 2003). Therefore, the lack of

discernible detrimental effects to non-target insects from TC1507 demonstrated in the studies below strongly suggests that there should also be no effects to non-target insects from DAS-06275-8. Although, 6275 corn has higher Cry1F protein expression than poCry1F corn in plant tissues most likely to be exposed to the soil dwelling Collembola and earthworms (**Table 3**), the Collembola and earthworm studies conducted for poCry1F used concentrations of Cry1F protein that exceed those that would be seen from the proposed uses of either poCry1F or moCry1F corn. This suggests that the proposed uses of Cry1F protein in corn are not likely to have any measurable population effects on soil invertebrates including Collembola and earthworms.

iv. Honey Bee Studies [USEPA OPPTS 885.4380 [MRID No. 450415-03, 453078-05 (supplement)]]

Honey bee (*Apis mellifera*) larvae were fed Cry1F corn pollen and pure Cry1F protein in a capped honey bee brood cell study with normal larval development and emergence of healthy adult honey bees. There was no significant difference between treatment mortality and behavior change between the dosed and control replicates. The LC_{50} was greater than 64 ng Cry1F in 2 mg pollen /larva and 640 ng Cry1F protein /larva. The study showed 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. The study results suggest that there should be no discernible detrimental effects to honey bees from the proposed uses of Cry1F producing corn including the moCry1F hybrid.

v. Green lacewing, *Chrysoperla carnea* [USEPA OPPTS 885.4340 [MRID Nos. 450201-09, 453078-01 (supplement)]]

Green lacewing larvae (*Chrysoperla carnea*) were fed 15x the expected rate of Bt Cry1F protein found in 1507 corn pollen over a 13 day period. There were no mortalities or signs of toxicity due to feeding on Cry1F at these levels, and thus the NOEC was determined to be greater than 15x the concentration of Cry1F found in pollen. The LC_{50} was determined to be greater than 480 ppm a.i. (the test concentration). These data are corroborated by the abundance of adult and larval green lacewings found in the field census study submitted in support of the Herculex I registration (MRID No. 450201-13). The data do not show significant detrimental effects or risk to beneficial insects at Cry1F levels that will be encountered in the field use situation.

vi. Ladybird beetle, *Hippodamia convergens* [USEPA OPPTS 885.4340 [MRID Nos. 450201-10 & 453078-02 (supplement)]]

Adult ladybird beetles, *Hippodamia convergens*, were fed 15x the expected rate of Cry1F protein found in event TC1507 corn pollen with no mortality or signs of toxicity over a 29 day period. The NOEC was determined to be greater than 15x the concentration of Cry1F protein found in pollen and the LC_{50} was determined to be greater than 480 ppm a.i. (the test concentration). These data were also supported by the abundance of adult and larval ladybird beetles found in the field census study submitted in support of the Herculex I (TC1507) registration (MRID No. 450201-13). The data do

not show significant detrimental effects or risk to beneficial insects including beetles for the proposed uses of Cry1F producing corn including the 6275 hybrid.

vii. Parasitic Hymenoptera, *Nasonia vitripennis* [USEPA OPPTS 885.4340 [MRID Nos. 450201-11, 453078-03 (supplement)]]

Parasitic Hymenoptera, *Nasonia vitripennis,* were fed a concentration of Bt Cry1F protein at 10x the expected rate found in TC1507 corn pollen with no mortality or signs of toxicity over a 12 day period. The NOEC was determined to be greater than 10x the concentration of Cry1F found in pollen and the LC_{50} was determined to be greater than 320 ppm a.i. (the test concentration). These data were further supported by the abundance of parasitic wasps found in the field census study submitted in support of the Herculex I registration (MRID No. 450201-13). Therefore, no adverse effects to parasitic wasps are expected from field exposure to corn producing Cry1F protein including moCry1F.

viii. Monarch butterfly, *Danaus plexippus* [USEPA OPPTS 885.4340 [MRID No. 451311-02]]

Neonate monarch butterfly, *Danaus plexippus*, larvae were fed a ≤10,000 ng/mL diet dose of Cry1F protein for seven days. 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, the Agency concluded that Cry1F protein will not pose a risk to monarchs.

ix. Collembola, *Folsomia candida* [USEPA OPPTS 885.4340 [MRID No. 450201-07]]

Collembola, *Folsomia candida*, feed on decaying plant material in the soil and thus would likely be exposed to corn plants containing Cry1F. The effects of Cry1F protein on *F. candida* was investigated in a study where this species was fed test concentrations of Cry1F protein every two to three days for 28 days representing 79x, 388x, and 1560x that which would be encountered in the field with no observable treatment mortality or behavior change. These results indicate that the proposed uses of Cry1F protein in corn are not likely to have any measurable population effects on soil invertebrates including Collembola.

x. Earthworms, *Eisenia fetida* [OECD Guideline 207 [MRID Nos. 450201-06, 453078-04 (supplement)]]

Earthworms, *Eisenia fetida*, fed 2.26 mg Cry1F/kg dry soil, representing up to 100X the estimated concentration present in the top six inches of an acre of soil following the incorporation of 25,000 senescent corn plants did not have adverse effects. Therefore, no adverse effects to earthworms or other beneficial soil invertebrates are expected from field exposure to corn producing Cry1F protein including event DAS-06275-8.

xi. Non-target Insect Field Survey [Guideline154-35 [MRID No. 450201-13]]

Based on recommendations by the October 2000 Scientific Advisory Panel (SAP) (Report No. 2000-07) (SAP, 2001), the EPA sent out a letter on October 15, 2001 to all Bt corn registrants requesting that they submit confirmatory data from field studies evaluating potential impacts of Bt on non-target invertebrates. The letter extended Bt corn registrations to October 2008 during which time, Bt corn registrants were expected to provide confirmatory field data regarding potential impacts on non-target invertebrates. In response to the EPA's request for field data, the Agricultural Biotechnology Stewardship Committee (ABSTC), Non-target Organism Subcommittee (NTO) submitted summaries and reports of non-target field survey studies in Bt corn on March 15, 2002. **Table 4** summarizes the Cry1F field survey information that was submitted. The Agency concluded that the information submitted indicated that the test material did not produce unexpected adverse effects on the non-target species on the basis of one growing season (Rose, 2003; MRID 456520-01; MRID 456480-01). Additional data will be collected and a final report is due to the Agency in late 2005.

Table 4. Field surveys of non-target invertebrates in *Bt* Cry1F corn.

Study	# Years	Study Design	Taxa Collected	Top Line Results
Field survey of beneficial arthropods associated with *Bt* Cry1F maize (Higgins 1999) **MRID No. 450201-13**	1 year 1999	**TC1507 & 1360:** 2 treatments: Cry1F and non-*Bt* near isoline; plot size: 28 x 4 m; 1 site (Iowa) 4 reps; sampling: visual and sticky trap observations before, during, and after anthesis	lady beetles, predacious beetles, lacewings, insidious flower bugs, assassin bugs, damsel bugs, parasitic wasps, damsel or dragonflies, and spiders.	Cry1F maize lines had no effect on the presence of beneficial arthropods compared with non-*Bt* near isolines; generally, TC1507 showed larger numbers of beneficial insects
Field study of non-target arthropods associated with *Bt* var. aizawai Cry1F maize **MRID No. 456520-01**	1 year (2000)	**TC1507:** 3 treatments: Cry1F, non-*Bt* near isoline, and non-*Bt* near isoline treated with foliar insecticide (lamba-cyhalothrin); plot size: 24 x 6 m; 1 site (France) 4 reps; sampling: visual observations 7X during growing season	thrips, insidious flower bugs, leafhoppers	Cry1F showed no significant impact on the non-target arthropods. Insecticide treatment significantly reduced non-target arthropod populations
Field survey of non-target arthropods associated with *Bt* Cry1F maize **MRID No. 456480-01**	1 year (2001)	**TC1507:** 2 treatments: Cry1F and non-*Bt* near isoline; plot size: 3.5 A; 1 site (Iowa); sampling: visual and sticky trap observations before, during, and after anthesis	community census	Abundance of key taxa showed no consistent reduction in the Cry1F field, although fewer parasitic hymenoptera were observed.

The field study conducted in 1999 by Higgins (MRID No. 450201-13) and reviewed by the EPA (EPA 2001b) for the registration of Herculex I (poCry1F) counted: ladybird beetles (*Cycloneda munda* & *Coleomegilla maculata*), predacious ground beetles (Carabidae), brown lacewings (Hemerobiidae), green lacewings (*Chrysoperla plorabunda*), minute pirate bugs (*Orius insidiosus*), assassin bugs (Reduviidae), damsel bugs (Nabidae), parasitic wasps (Ichneumonidae and Braconidae, damselflies and dragonflies (Odonata), and spiders (Arachnida). Data included counts of adult and larval lady beetles and lacewings. Results from the study indicated that the transgenic

corn lines TC1507 and 1360 did not adversely affect the number of beneficial arthropods in the field. In general, line TC1507 showed larger numbers of beneficial insects. The field census study adequately addressed potential concerns for Cry1F protein expressed in corn to non-target insect populations. However, the Agency recommended that the monitoring continue into the first few years of commercial use of Cry1F corn crops in order to confirm the single season "no effects" findings and to gather long-range non-target insect effects and abundance data.

b. Hazard Characterization for Aquatic Wildlife

There is no evidence for sensitivity of aquatic (including endangered) species to anti-lepidopteran Cry proteins. Aquatic species toxicity studies with lepidopteran-active Cry proteins have not revealed hazard for fish or invertebrates exposed to either corn pollen or to bacterially expressed Cry protein. Furthermore, aquatic exposure from Bt crops is extremely small.

i. Freshwater Fish Hazard Assessment [Fish Acute Toxicity Test, Freshwater and Marine USEPA OPPTS 850.1075 [MRID Nos. 450442-01 & 460193-06]]

The requirement for a freshwater fish static renewal toxicity study was waived for the registration of Herculex I containing poCry1F primarily based on the lack of substantial exposure of fish to the Bt Cry proteins produced in Bt crops (USEPA 2001). However, Dow AgroSciences has submitted this study in support of the event DAS-06275-8 registration. Juvenile rainbow trout (*Onchorhynchus mykiss*) were fed a standard fish diet containing 100 mg Cry1F ICP a.i./kg (100 ppm) of diet for eight days with no mortality or sublethal effects. The LD_{50} was determined to be greater than 100 mg a.i./kg of diet. However, the actual concentration of the test material in the diet was not determined and therefore this study is supplemental.

ii. Aquatic Invertebrate Hazard Assessment [Aquatic Invertebrate Acute Toxicity Testing, Tier I USEPA OPPTS 850.1010 [MRID No. 450201-08]]

Toxicity studies using corn pollen containing Cry1F protein were conducted with *Daphnia magna* (EPA, 2001b). The no-mortality concentration and NOEC were greater than 100 mg a.i./L. There were no overt signs of toxicity for daphnids exposed to 100 mg Bt Cry1F pollen/L and the amount of pollen tested was considered to well exceed field exposure. These results suggest that Cry1F proteins expressed in pollen do not pose a hazard to *D. magna* and aquatic invertebrates in general.

iii. Estuarine and Marine Animal Hazard Assessment [Fish Acute Toxicity Test, Freshwater and Marine USEPA OPPTS 850.1075 [No MRID assigned]]

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

c. Hazard Characterization for Terrestrial and Aquatic Plants [Non-target Plant Studies, Tier I USEPA OPPTS 885.4300 [No MRID assigned]]

The plant toxicity studies were waived for this product because the active ingredient is an insect toxin (Bt endotoxin) that has never shown any toxicity to plants.

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, including the monarch butterfly, a "may effect" situation for endangered land and aquatic species (except possibly some Lepidoptera species) is not anticipated. 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. 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 United States clearly indicated that any potential concern regarding range overlap with corn production was mainly restricted to the Karner blue and possibly the Mitchell satyr butterflies *(Neonympha mitchellii mitchellii)*. After careful review of the available data, the EPA determined that 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, indicated that no exposure to harmful levels of Cry1F protein containing pollen would take place. Therefore, the EPA has determined that the Cry1F protein expressed in corn is not likely to adversely affect listed species. See the EPA's BRAD for the *Bacillus thuringiensis* (Bt) Plant-Incorporated Protectants for more details (EPA 2001b).

D. INSECT RESISTANCE MANAGEMENT

The Agency completed a full reassessment of the insect resistance management strategies for both Cry1Ab and poCry1F corn (EPA 2001b). The February, 1998 FIFRA Scientific Advisory Panel (SAP) Subpanel on *Bacillus thuringiensis* (Bt) Plant-Pesticides and Resistance Management determined that a high dose/refuge strategy is necessary to mitigate resistance of stalk boring Lepidoptera in Bt corn (meeting held on February 9-10, 1998. Docket # OPPTS-00231). The SAP determined that a high dose (defined as 25× the dose necessary to kill all susceptible insects) should be verified by two of five techniques outlined in the final report (SAP, 1998). A summary of the Agency's IRM review for poCry1F is found in EPA's Biopesticides Registration Action Document for *Bacillus thuringiensis* Plant-Protectants (EPA 2001b). The Agency's assessment of the field efficacy and adequacy of the IRM strategy for 6275 corn is found in Hill (2004). A brief summary of the key findings for both TC1507 and DAS-06275-8 IRM is found below.

Pests susceptible to the Cry1F protein from events TC1507 and DAS-06275-8 include: European corn borer (ECB), *Ostrinia nubilalis* (Huebner); southwestern corn borer (SWCB), *Diatraea grandiosella* (Dyar); fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith); black cutworm (BCW), *Agrotis ipsilon* (Hufnagel); western bean cutworm (WBCW), *Richia albicosta* (Smith), and corn earworm (CEW), *Helicoverpa zea* (Boddie). A high dose has been demonstrated for ECB and a high level of efficacy was found for SWCB, FAW, and BCW. Unlike currently registered Cry1Ab field corn hybrids, Cry1F is highly efficacious against the BCW and FAW.

1507 corn was shown to provide a high dose (using two SAP methods #s 4 &5) against ECB (EPA 2001b). A high dose was measured using a *single* approach (SAP method #4- survey TC6275 corn. 6275 corn caused greater than 99.99% mortality of ECB in the field. If evaluated independent of other available information, the high dose study would be considered supplemental (upgradable to acceptable upon submission of verification of high dose by a second EPA approved method). However, the following data provide additional lines of evidence to sufficiently support event DAS-06275-8 as producing a high dose of protein to control ECB as defined by the SAP: 1) Field efficacy data for 6275 corn in comparison to 1507 corn (see Hill, 2004) show that the performance of DAS-06275-8 is statistically equivalent to that of TC1507 and that both events are able to resist infestation of multiple lepidopteran pest species. Furthermore, 1507 corn was shown to provide a high dose using two SAP methods (#s 4 &5) against ECB (EPA 2001b). Because event TC1507 has been determined to provide a high dose for ECB and DAS-06275-8 has been shown to have field efficacy equivalent to that of TC1507, it is reasonable to assume that DAS-06275-8 also provides a high dose for at least ECB. 2) An analysis of the comparative expression of Cry1F protein in 6275 and 1507 corn at different plant growth stages (Zabik *et al.* 2003) shows a significantly increased amount of Cry1F protein in 6275 stalks at the R1 stage as well as increased Cry1F in leaves, whole plants, and forage in 6275 compared to 1507 (see **Table 3**). Because DAS-06275-8 has greater expression of Cry1F than TC1507 and TC1507 has been shown to provide high dose for ECB; it should follow that DAS-06275-8 also provides a high dose. The IRM requirements that were mandated by EPA for 1507 corn (EPA 2001b) are also applicable to 6275 corn.

E. BENEFITS

EPA has reviewed the public interest document for Cry1F corn (Event DAS-06275-8) (Matten, 2005c) and Cry1F corn (Event TC1507) (EPA 2001a and b). Maize-optimized Cry1F-protected corn will solidify and extend the benefits of insecticide use reduction that have been established for plant-optimized Cry1F-protected corn. Just as for 1507 corn, 6275 corn is comparatively less risky to health or the environment than currently registered pesticides and the expected benefits (including economic benefits) from the use of the new active ingredient are greater than those of alternative registered pesticides and other available non-chemical techniques.

Summary of Public Interest Finding Criteria

The criteria for a determination as to whether registration of a pesticide chemical is in the public interest are set forth in a Federal Register notice dated 3-5-1986 volume 51, No.43 (OPP-32500; FRL-2977-2) Conditional Registration of New Pesticides. There is a presumption that registration of a pesticide chemical is in the public interest if one of the following criteria is met: (i) the use is for a minor crop; (ii) the use is a replacement for another pesticide that is of continuing concern to the Agency; (iii) the use is one for which an emergency exemption under FIFRA Section 18 has been granted for lack of an alternative pest control method, or (iiii) the use is against a pest of public health significance. Notwithstanding whether a registration of a pesticide chemical may be presumed to be in the public interest, EPA may determine that such a registration is in the public interest on the basis of the following criteria: (i) there is a need for the new chemical that is not being met by currently registered pesticides; (ii) the new pesticide is comparatively less risky to health or the environment than currently registered pesticides; or (iii) 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.

USDA/APHIS Environmental Assessment for Maize-optimized Cry1F-protected Maize

USDA/APHIS prepared an environmental assessment that addressed questions pertinent to the risk to the human environment, including plant pest risks, that could potentially result from an APHIS determination of nonregulated status under 7 CFR Part 340.6 for corn line 6275 and its progeny and their subsequent cultivation in the United States and its territories (USDA/APHIS, 2004). It also considered restrictions placed on the cultivation of this line stipulated in the pesticide registration granted by the EPA. APHIS came to the following conclusions following its evaluation:

"1. Line 6275 corn exhibits no plant pathogenic properties. Although DNA from pathogens were used in its development, these plants are not infected by these organisms, nor can these plants incite disease in other plants.
2. Line 6275 corn is no more likely to become a weed than insect or herbicide tolerant corn that is currently being cultivated. Corn is not a weed, and there is no reason to believe that

the introduced genes would enable corn to become a weed pest.
3. Introgression from line 6275 corn into wild plants in the United States and its territories is extremely unlikely. Potential introgression from line 6275 corn into wild relatives is not likely to increase the weediness potential of any resulting progeny nor adversely effect genetic diversity of related plants any more than would introgression from traditional corn hybrids.
4. Line 6275 corn is substantially equivalent in whole plant forage composition and in kernel composition, quality and other characteristics to nontransgenic corn and should have no adverse impact on raw or processed agricultural commodities.
5. Line 6275 corn will not have a significant adverse impact on nontarget organisms, including those beneficial to agriculture; and it will not affect threatened or endangered species.
6. Compared to current agricultural practices, cultivation of line 6275 corn should not reduce the ability to control insects or weeds in corn or other crops."

BENEFITS ASSESSMENT

Benefit Claims Made in Maize-optimized Cry1F-protected Corn Event TC6275 Public Interest Document (Zabik et al., 2003; MRID# 460193-12)

Dow AgroSciences (Dow) believes that the event DAS-06275-8 Cry1F-protected corn is clearly in the public interest and provides data to support the following claims:

1. Event DAS-06275-8 corn provides highly efficacious control of key Lepidopteran pests of field corn

2. 6275 corn has the potential to provide comparable or superior pest control to existing commercial Bt corn products for key corn pests and affords a broader spectrum of pest control than do corn products expressing the Cry1A(b) insect resistance trait.

3. The use of 6275 corn is expected to reduce the use of chemical insecticides.

4. Yields of 6275 corn varieties will be significantly greater than the yields of the non-Bt varieties.

5. Economic models show that 6275 corn maximizes economic benefits as compared to the application of conventional chemical insecticides to control Lepidopteran pests that are susceptible to Cry1F.

6. 6275 corn represents a competitive choice for insect control.

7. 6275 corn will solidify and extend the benefits of insecticide use reduction that have been established for 1507 corn.

8. Cry1F expressed in corn poses no foreseeable risks to human health or the environment.

Field Efficacy

Field efficacy trials were conducted over multiple locations and years. EPA reviewed the 6275 corn field efficacy data submitted by Dow AgroSciences (Babcock and Bing, 2003) and found these data to be "acceptable" (see EPA review, Hill, 2004). Dow has submitted sufficient field efficacy data to demonstrate that event DAS-06275-8 offers excellent control of European corn borer (*Ostrinia nubilalis* (Huebner), ECB), southwestern corn borer (*Diatraea grandiosella* (Dyar), SWCB), fall armyworm (*Spodoptera frugiperda* (J.E. Smith), FAW), black cutworm (*Agrostis ipsilon* (Hufnagel), BCW), Western bean cutworm (*Richia albicosta* (Smith), WBCW), and suppression of the corn earworm(*Helicoverpa zea* (Boddie), CEW). 6275 corn performed comparably to 1507 ("Herculex I") and showed significantly less infestation than the non-Bt isogenic hybrid. No additional data are required at this time.

Comparative Product Performance

Dow submitted an analysis of the comparative efficacy of event DAS-06275-8 corn against several commercial standards (conventional and transgenic) for insect control in corn. A no treatment (no Cry1F) control was also included. Based on this analysis, event DAS-06275-8 provides comparable performance relative to TC1507. 6275 corn performed better or comparably with the commercial standards against ECB, SWCB, BCW, FAW, CEW, and WBCW.

Yield and Economic Benefits

The primary economic benefit of corn hybrids containing Cry1F insect resistance trait (TC1507 and DAS-06275-8) is the protection of yield. At the individual farm level, there will be cost savings from reduced field scouting and applying fewer chemical insecticides. The two factors that drive a farmer to choose a Bt corn hybrid versus conventional hybrids treated with chemical insecticides are : 1) the higher level of field efficacy that Bt corn hybrids, such as Cry1F corn hybrids, offer in comparison to current chemical insecticides and 2) the "insurance" factor of a Bt corn hybrid as a prophylactic control measure. Both of these factors result in potential yield benefits to the farmer.

Yield and Agronomic Characteristics

Previously, EPA concluded that Event TC1507 corn hybrids expressing Cry1F protein were competitive with other *Bt* hybrids as well as with non-*Bt* corn varieties (EPA, 2001b). Event DAS-06275-8 corn hybrids expressing Cry1F were also competitive with 1507 hybrids as well as non-*Bt* corn varieties.

Agronomic performance traits of 6275 hybrids were compared to isogenic hybrids containing event TC1507 and isogenic hybrids containing no transgenic trait in field trials for early (11 locations) and late (15 locations) season hybrids in 2002. The comparison of both late and early maturity hybrids found no statistically significant differences within either maturity group among the 6275 hybrid, the 1507 hybrid, and non-transgenic isogenic hybrid for grain density, plant stature, emergence vigor,

root lodging, and dropped ears (see Tables 5 and 6 in Zabik et al., 2003). There were no statistically significant yield differences between either early or late maturity comparisons of 6275 and 1507 hybrids. Both the early maturity 6275 and 1507 hybrids were significantly higher yielding than the early maturity non-transgenic isogenic hybrid. This is most likely due to the protection offered by Cry1F against European corn borer-induced yield loss. Both early and late maturity 6275 hybrids had significantly better top integrity scores than the non-transgenic hybrids. Although there were some statistically significant differences found in other parameters, these differences were not considered to biologically or commercially significant. Based on this analysis, there would be no expectation of increased likelihood of weediness due to the presence of the *cry*1F transgene in event DAS-06275-8.

Economic Benefits

EPA has previously analyzed the economic benefits resulting from Cry1F-protected corn (Event TC1507) (EPA, 2001b). At product maturity, grower benefits of Cry1F-protected corn are estimated to be between $28 and $81 million per year on 7.3 to 12.5 million acres of field corn. The range depends upon the technology fee, from $7.50 to $13.13/acre. Bt-related costs are assumed to be $10/acre. These costs cover refuge requirements and marketability concerns and apply to situations where Cry1F replaces chemical control or no control. Acres at risk are estimated to be 25 million acres, based on the states affected and the extent of area infested. Grower benefits could vary by an average of $3.90/acre to $6.51/acre. The very wide range is due to the wide range of the proposed technology fee. It should be noted that these annual benefits would occur at product maturity, or 3 to 5 years after commercialization. The analysis does not consider possible stacked products which offer multiple protections and efficiencies, the effect of new competitor products, or the impact of increased competition on overall market equilibrium conditions. Increased competition should offer growers more choice and lower the cost of pest control. The benefits are the incremental improvement to grower profits compared to current practice. All costs are eventually passed along to consumers in the long run, but this analysis did not deal with when that would occur.

The economic benefit to the grower who plants corn varieties containing Event DAS-06275-8 was evaluated on the basis of data on yield and efficacy as reported in Zabik et al. (2003) using the TC1507 Value Calculator. Three scenarios using both early and late maturity hybrids were used to illustrate the economic benefit associated with Event DAS-06275-8 (see Table 7, p. 33, in Zabik et al. 2003). As summarized in Table 7 of Zabik et al. (2003), the value of the Event TC6275 corn ranged from $40 to $98 per acre. The economic benefit of ECB protection accounts from from $24 to $81 per acre of this total with an added benefit of from $16 to $17 per acre for BCW protection.

Marketing Issues

DAS-06275-8 and TC1507 are positioned to compete against the following chemical alternatives: bifenthrin, carbofuran, chlorpyrifos, cyhalothrin-lambda, permethrin, fipronil, tebuprimiphos/cyluthrin, tefluthrin, terbufos, and zeta-cypermethrin and against the Cry1Ab-protected corn hybrids. The registered chemical alternatives commonly used to treat the target pest

complex protected by Cry1F are restricted use for the most part. They have precautionary label statements such as extremely toxic to fish and aquatic organisms, wildlife and require protective clothing for workers. The specific organophosphate and pyrethroid pesticides likely to be replaced are ranked in the top 15 of all pesticides with respect to reported incidents of mortality to non-target wildlife. Many of these products also control corn rootworm, which is the most significant pest of corn and is frequently treated along with the lepidopteran target pest complex of Cry1F. Cry1F performance data have shown performance equal to or better than any of the conventional chemical alternatives or other Bt corn hybrids against ECB, BCW, SWCB, FAW, WBCW, and CEW. Growers may be more likely to choose Cry1F protected corn due to better product performance and broader spectrum of control. Cry1F protected corn is also expected to be economical on some unprotected fields and provide insurance against the risk of crop loss and the need to replant. But without rootworm protection, the use of Cry1F to reduce conventional pesticide use is somewhat limited.

Human Health and Environmental Risks and Benefits

The Agency's human health and environmental safety assessments attest to the safety of the Cry1F protein (from both event TC1507 and DAS-06275-8) that it is expected to pose no unreasonable adverse effects to human health or the environment (Matten, 2005a and b; Hill, 2005; EPA 2001 a and b). Based on the Agency's previous evaluation of the benefits of Event TC1507 (EPA, 2001a and b), Event DAS-06275-8 can also substantially reduce the health and environmental risks associated with the use of traditional chemical insecticides. Cry1F-protected corn varieties will potentially decrease the reliance on conventional pesticides when used as part of an integrated pest management program. Reductions in the use of conventional pesticides would eliminate the need to transport, mix, apply, and dispose of these pesticides, reduce spray-drift and run-off associated with some of the registered alternatives, and reduce potential adverse effects to non-target organisms. Increased use of Cry1F-protected corn would also improve worker protection as compared to chemical insecticides.

The data submitted show that 1507 and 6275 corn produce the same Cry1F protein and therefore have the same expected toxicity to target pests. Data also demonstrate that Cry1F is not heat labile (MRID 452748-01), is rapidly digested in simulated gastric fluid (MRID 447149-03), and does not share any amino acid sequence similarity to known allergens (MRID 449717-01). These data indicate there is no likely potential for the Cry1F protein to be a food allergen.

Expression data reveal that 6275 plants express lower concentrations of Cry1F protein in pollen and grain than 1507 plants (1/6 and1/2, respectively) while 6275 plants express the Cry1F protein three to eight times greater in the leaf than the 1507 plants (Zabik et al. 2003, see p. 36, Table 8). Increased expression in the stalks and leaves should decrease the risk of resistance development by borers (a higher, high dose) whilst the decreased expression in pollen and grain should decrease the non-target exposure to Cry1F expressed in corn (Zabik *et. al* 2003).

Similarly, testing with bacterially prepared Cry1F protein at levels greatly exceeding those found in maize optimized plants resulted in no effect with several beneficial species including the monarch butterfly. Additionally, field monitoring for effects of poCry1F corn on non-target insects confirmed the absence of adverse effects to non-target organisms (MRID 450201-13, see Table 1). Data were also provided regarding the rapid soil degradation of poCry1F protein, approximately 3.13 days (MRID 450201-05), although the Agency required additional data to study long-term soil degradation (EPA, 2001b).

F. REFERENCES

Babcock, J.M. and J. Bing. 2003. Field efficacy of Maize-optimized Cry1F (TC6275) for the control of Lepidoptera pests of corn. Unpublished study submitted by Dow AgroSciences LLC. MRID No. 460630-04.

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U.S. Department of Agriculture (USDA), Animal Plant Health Inspection Service (APHIS). 2004. Approval of Mycogen Seeds c/o Dow AgroSciences LLC Request (03-181-01p) Seeking Extension of Determination of Non-regulated Status for Bt Cry1F Insect Resistant, Glufosinate Tolerant Corn Line 6275. Finding of no significant impact. October 20, 2004.

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U.S. Environmental Protection Agency (EPA) 2001b. Biopesticides Registration Action Document: *Bacillus thuringiensis* (Bt) Plant-incorporated Protectants (October 15, 2001). http://www.epa.gov/pesticides/biopesticides/pips/bt_brad.htm.

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Zabik, J. M., J. D. Wolt, D.J. Borgmeier, N. Storer. 2003. Public interest document for maize-optimized Cry1F-protected corn event TC6275. GH-C 5659. MRID 460193-12.

III. Terms and Conditions of Registration

The following terms and conditions of registration are required:

1) The subject registration will automatically expire on midnight October 15, 2008.

2) The subject registration will be limited to the use of *Bacillus thuringiensis* var. *aizawai* strain PS811 Cry1F insecticidal crystal protein and the genetic material necessary for its production (plasmid insert PHP12537) in Cry1F Event DAS-06275-8 corn.

3) Submit/cite all data required for registration of your product under FIFRA § 3(c)(5) when the Agency requires registrants of similar products to submit such data.

4) Submit production information for this product to Mr. Owen Beeder of Office of Pesticide Programs, Registration Division (mail code 7505C) for the fiscal year in which this product is conditionally registered, in accordance with FIFRA § 29. The fiscal year begins October 1 and ends September 30. Production information will be submitted to the Agency no later than December 15, following the end of the preceding fiscal year.

5) The protocol for the Independent Lab Validation of analytical method GRM02.13 "Determination of Cry1F Insecticidal Crystal Protein in corn grain by Enzyme Linked Immunosorbant Assay" does not include a transgenic moCry1F treatment to substantiate the lack of interference posed by transgenic plant expression of Cry1F. Modify the protocol and submit to the EPA appropriate documentation to demonstrate that this method works to quantify Cry1F expressed in transgenic moCry1F corn on or before November 15, 2005.

6) Submit to the EPA laboratory (Ft. Meade, MD) methodology and/or reagents necessary for validation of a moCry1F analytical method within 6 months of the date of registration (under OPPTS Guidelines 860.1340). The extraction and detection method and independent third party laboratory validations as described for moCry1F protein appear to be adequate for analysis of moCry1F protein in corn grain. However, this method must be validated by both an independent laboratory and the EPA Biological and Economic Analysis Division laboratory before it can be considered a valid method.

7) Submit confirmatory testing of moCry1F protein levels in soil under a range of conditions typical of *Bt* corn cultivation. EPA requires Mycogen Seeds c/o Dow AgroSciences LLC in cooperation with other registrants to submit test protocols before the studies are actually conducted. In general, the Agency anticipates that soils would be sampled from fields where *Bt* corn has been grown continuously for at least 3 years compared with fields where no *Bt* crop has been grown. These paired fields would include several locations throughout the corn growing area of the US representing different soil and climatic variations. The Agency anticipates that samples would need to be taken 2 or 3 times during the growing season. A protocol was approved for poCry1F in 2002. Submit a final report to the Agency for moCry1F by March 15, 2008.

8) Submit confirmatory field data for possible impacts of moCry1F on non-target insects. Preliminary data for poCry1F corn were provided to the Agency on March 14, 2002 and found to be acceptable. Submit a final report for moCry1F to the Agency by March 15, 2008.

The following registration requirements and conditions shall not require any action by Mycogen Seeds c/o Dow AgroSciences LLC unless and until Mycogen Seeds c/o Dow AgroSciences LLC commercializes moCry1F corn in the United States. **The term "commercialization" shall**

mean the sale of moCry1F corn seed to one or more growers for purposes of growing a commercial grain corn crop in the United States.

You must commit to do the following Insect Resistance Management Program upon commercialization:

1] Requirements relating to creation of a non-*Bt* corn or non-lepidopteran resistant *Bt* corn refuge in conjunction with the planting of any acreage of *Bt* corn;

2] Requirements for the registrant to prepare and require *Bt* corn users to sign "grower agreements" which impose binding contractual obligations on the grower to comply with the refuge requirements;

3] Requirements for the registrant to develop, implement, and report to EPA on programs to educate growers about IRM requirements;

4] Requirements for the registrant to develop, implement, and report to EPA on programs to evaluate and promote growers' compliance with IRM requirements;

5] Requirements for the registrant to develop, implement, and report to EPA on programs to evaluate whether there are statistically significant and biologically relevant changes in target insect susceptibility to Cry1F protein in the target insects;

6] Requirements for the registrant to develop, and if triggered, to implement a "remedial action plan" which would contain measures the registrant would take in the event that any insect resistance was detected as well as to report on activity under the plan to EPA;

7] Submit annual reports on sales, IRM grower agreements results, compliance, and educational program on or before January 31st each year after commercialization.

a. Refuge Requirements

1) Corn-Belt Refuge Requirements

For lepidopteran resistant *Bt* field corn grown outside cotton-growing areas (e.g., the Corn Belt), grower agreements (also known as stewardship agreements) will specify that growers must adhere to the refuge requirements as described in the grower guide/product use guide and/or in supplements to the grower guide/product use guide.

- Specifically, growers must plant a structured refuge of at least 20% non-*Bt* corn or non-lepidopteran resistant *Bt* corn that may be treated with insecticides as needed to control

lepidopteran stalk-boring and other pests.

- Refuge planting options include: separate fields, blocks within fields (e.g., along the edges or headlands), and strips across the field.

- External refuges must be planted within ½ mile (1/4 mile or closer preferred).

- When planting the refuge in strips across the field, refuges must be at least 4 rows wide, preferably 6 rows wide.

- Insecticide treatments for control of ECB, CEW, Southwestern corn borer (SWCB), fall armyworm (FAW), black cutworm (BCW), and western bean cutworm (WBCW) 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.

2) Cotton-Growing Area Refuge Requirements for *Bt* Corn

For *Bt* field corn grown in cotton-growing areas, grower agreements (also known as stewardship agreements) will specify that growers must adhere to the refuge requirements as described in the grower guide/product use guide and/or in supplements to the grower guide/product use guide.

- Specifically, growers in these areas must plant a structured refuge of at least 50% non-*Bt* corn or non-lepidopteran resistant *Bt* corn that may be treated with insecticides as needed to control lepidopteran stalk-boring and other pests.

- Refuge planting options include: separate fields, blocks within fields (e.g., along the edges or headlands), and strips across the field.

- External refuges must be planted within ½ mile (1/4 mile or closer preferred).

- When planting the refuge in strips across the field, refuges must be at least 4 rows wide, preferably 6 rows wide.

- Insecticide treatments for control of ECB, CEW, Southwestern corn borer (SWCB), fall armyworm (FAW), black cutworm (BCW), and western bean cutworm (WBCW) 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 or non-lepidopteran resistant *Bt* corn refuges.

- Cotton-growing areas include the following states: Alabama, Arkansas, Georgia, Florida, Louisiana, North Carolina, Mississippi, South Carolina, Oklahoma (only the counties of Beckham, Caddo, Comanche, Custer, Greer, Harmon, Jackson, Kay, Kiowa, Tillman, Washita), Tennessee (only the counties of Carroll, Chester, Crockett, Dyer, Fayette, Franklin, Gibson, Hardeman, Hardin, Haywood, Lake, Lauderdale, Lincoln, 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 Dinwiddie, Franklin City, Greensville, Isle of Wight, Northampton, Southampton, Suffolk City, Surrey, Sussex) and Missouri (only the counties of Dunkin, New Madrid, Pemiscot, Scott, Stoddard). The correct list of counties must be in the first grower guide.

b. Grower Agreements

1] Persons purchasing the moCry1F *Bt* corn products must sign a grower agreement. The term "grower agreement" refers to any grower purchase contract, license agreement, or similar legal document.

2] The grower agreement and/or specific stewardship documents referenced in the grower agreement must clearly set forth the terms of the current IRM program. By signing the grower agreement, a grower must be contractually bound to comply with the requirements of the IRM program.

3] Mycogen Seeds c/o Dow AgroSciences LLC must establish by the year of commercialization, a system which is reasonably likely to assure that persons purchasing the *Bt* corn product will affirm annually that they are contractually bound to comply with the requirements of the IRM program.

4] At least 30 days prior to commercialization, Mycogen Seeds c/o Dow AgroSciences LLC must submit to EPA a copy of its grower agreement and any specific IRM documents referenced in the grower agreement. If Mycogen Seeds c/o Dow AgroSciences LLC wishes to change any part of the grower agreement or any specific stewardship documents referenced in the grower agreement that would affect either the content of the IRM program or the legal enforceability of the provisions of the agreement relating to the IRM program, thirty days prior to implementing a proposed change, the registrant must submit to EPA the text of such changes to ensure that it is consistent with the terms and conditions of the amendment.

5] The registrant must establish a system which is reasonably likely to assure that persons purchasing the *Bt* corn sign grower agreement(s), and must provide by January 31st, of the first

year after the commercialization of moCry1F corn a written description of that system.

6] The registrant shall maintain records of all *Bt* moCry1F corn grower agreements for a period of three years from December 31st of the year in which the agreement was signed.

7] Beginning on January 31st after the first year of commercialization and annually thereafter, the registrant shall provide EPA with a report showing the number of units of its *Bt* corn seeds sold or shipped and not returned, and the number of such units that were sold to persons who have signed grower agreements. The report shall cover the time frame of the twelve-month period covering the prior August through July. Note: if the first year of commercialization is 2006, the first report shall contain the specified information for the time frame starting with the date of registration and ending July 31, 2007.

8] The registrant must allow a review of the grower agreements and grower agreement records by EPA or by a State pesticide regulatory agency if the State agency can demonstrate that confidential business information, including names, personal information, and grower license number, will be protected.

c. IRM Education and IRM Compliance Monitoring Programs

1] Mycogen Seeds c/o Dow AgroSciences LLC must design and implement a comprehensive, ongoing IRM education program designed to convey to *Bt* corn users the importance of complying with the IRM program. The program shall include information encouraging *Bt* corn users to pursue optional elements of the IRM program relating to refuge configuration and proximity to *Bt* corn fields. The education program shall involve the use of multiple media, e.g. face-to-face meetings, mailing written materials, EPA reviewed language on IRM requirements on the bag or bag tag, and electronic communications such as by Internet, radio, or television commercials. Copies of the materials will be provided to EPA for its records. The program shall involve at least one written communication annually to each *Bt* corn user separate from the grower technical guide. The communication shall inform the user of the current IRM requirements. Mycogen Seeds c/o Dow AgroSciences LLC shall coordinate its education programs with educational efforts of other registrants and other organizations, such as the National Corn Grower Association and state extension programs.

2] Annually after commercialization, the registrant shall revise, and expand as necessary, its education program to take into account the information collected through the compliance survey required under paragraph 6] and from other sources. The changes shall address aspects of grower compliance that are not sufficiently high.

3] Beginning January 31st, of the first year after commercialization of moCry1F corn and annually thereafter, the registrant must provide EPA any changes to its grower education

activities as part of the overall IRM compliance assurance program report. No separate grower education report is needed if the registrant submits a report in connection with other *Bt* registrants. The required features of the compliance assurance program are described in paragraphs 4]-15] below.

4] The registrant must design and implement an ongoing IRM compliance assurance program designed to evaluate the extent to which growers purchasing its *Bt* corn product are complying with the IRM program and that takes such actions as are reasonably needed to assure that growers who have not complied with the program either do so in the future or lose their access to the *Bt* corn product. The registrant shall coordinate with other registrants in designing and implementing its compliance assurance program. The registrant must prepare and submit by January 31st, of the first year after commercialization a written description of their compliance assurance. Other required features of the program are described in paragraphs 5] - 15] below.

5] The registrant must establish and publicize a "phased compliance approach," i.e., a guidance document that indicates how the registrant will address instances of non-compliance with the terms of the IRM program and general criteria for choosing among options for responding to any non-compliant growers. While recognizing that for reasons of difference in business practices there are needs for flexibility between different companies, all *Bt* corn registrants must use a consistent set of standards for responding to non-compliance. The options shall include withdrawal of the right to purchase *Bt* corn for an individual grower or for all growers in a specific region. An individual grower found to be significantly out of compliance two years in a row would be denied sales of the product the next year. Similarly, seed dealers who are not fulfilling their obligations to inform/educate growers of their IRM obligations will lose their opportunity to sell *Bt* corn.

6] The IRM compliance assurance program shall include an annual survey of a statistically representative sample of *Bt* corn growers conducted by an independent third party. The survey shall measure the degree of compliance with the IRM program by growers in different regions of the country and consider the potential impact of non-response. The sample size and geographical resolution may be adjusted annually, based upon input from the independent marketing research firm and academic scientists, to allow analysis of compliance behavior within the four ABSTC regions or between regions. The sample size must provide a reasonable sensitivity for comparing results across the U.S.

7] The survey shall be designed to provide an understanding of any difficulties growers encounter in implementing IRM requirements. An analysis of the survey results must include the reasons, extent, and potential biological significance of any implementation deviations.

8] The survey shall be designed to obtain grower feedback on the usefulness of specific educational tools and initiatives.

9] Prior to commercialization, the registrant shall provide a preliminary summary of its findings by November 15th of each year beginning with the year of commercialization and a final written summary of the results of the prior year's survey (together with a description of the regions, the methodology used, and the supporting data) to EPA by January 31 of each year following commercialization. The registrant shall confer with other registrants and EPA on the design and content of the survey prior to its implementation.

10] Annually after commercialization, the registrant shall revise, and expand as necessary, its compliance assurance program to take into account the information collected through the compliance survey required under paragraphs 6] through 8] and from other sources. The changes shall address aspects of grower compliance that are not sufficiently high. The registrants must confer with the Agency prior to adopting any changes.

11] Prior to commercialization, the registrant shall train its representatives who make on-farm visits with *Bt* corn growers to perform assessments of compliance with IRM requirements. In the event that any of these visits result in the identification of a grower who is not in compliance with the IRM program, the registrant shall take appropriate action, consistent with its "phased compliance approach" to promote compliance.

12] Prior to commercialization, the registrant shall establish a program for investigating legitimate "tips and complaints" that its growers are not in compliance with the IRM program. Whenever an investigation results in the identification of a grower who is not in compliance with the IRM program, the registrant shall take appropriate action, consistent with its "phased compliance approach."

13] If a grower, who purchases *Bt* corn for planting, was specifically identified as not being in compliance during the previous year, the registrant shall visit with the grower and evaluate whether that the grower is in compliance with the IRM program for the current year.

14] Beginning January 31st, and annually thereafter, registrant shall provide a report to EPA summarizing the activities carried out under their compliance assurance program for the prior year including changes to the grower education program, and the plans for the compliance assurance program during the current year. The report will include information regarding grower interactions (including, but not limited to on-farm visits, verified tips and complaints, grower meetings and letters), the extent of non-compliance, corrective measures to address the non-compliance, and any follow-up actions taken. The registrants may elect to coordinate information and report collectively the results of their compliance assurance programs.

15] The registrant and the seed corn dealers for the registrant must allow a review of

the compliance records by EPA or by a State pesticide regulatory agency if the State agency can demonstrate that confidential business information, including the names, personal information, and grower license number of the growers will be protected.

d. Insect Resistance Monitoring

The Agency is imposing the following conditions for this product upon commercialization:

1) After commercialization, Mycogen Seeds c/o Dow AgroSciences LLC will monitor for resistance and/or trends in increased tolerance for *Ostrinia nubilalis (*European corn borer), *Diatraea grandiosella (*Southwestern corn borer), and/or *Helicoverpa zea* (corn earworm). Sampling should be focused in those areas in which there is the highest risk of resistance development. The ABSTC has identified four regions for its compliance and monitoring programs. Sampling target for each insect pest will be at least 200 insects in any region where adoption of *Bt* corn exceeds 50% and the insect is a pest species in that region. Sampling target for each insect pest will be at least 100 insects in all other regions where the insect is a pest species in that region.

2) The registrant shall provide to EPA a description of its resistance monitoring plan by January 31st, of the first year after commercialization. The description shall include: sampling (number of locations and samples per locations), sampling methodology, bioassay methodology, standardization procedures, detection technique and sensitivity, and the statistical analysis of the probability of detecting resistance.

3) The registrant must follow up on grower, extension specialist or consultant reports of less than expected results or control failures for the target lepidopteran pests *Ostrinia nubilalis* (ECB), *Diatraea grandiosella (*SWCB), *Helicoverpa zea* (CEW/CBW), *Spodoptera frugiperda* (FAW), *Agrotis ipsilon* (BCW), and *Richia albicosta* (WBCW). The registrant will instruct its customers (growers and seed distributors) to contact them (e.g., via a toll-free customer service number) if incidents of unexpected levels of damage occurs from these target pests. The registrant will investigate all damage reports submitted to the company or the company's representatives. See Remedial Action Plans section below.

4) A report on results of resistance monitoring and investigations of damage reports must be submitted to the Agency annually by April 30th each year after the commercialization for the duration of the conditional registration.

e. Remedial Action Plans

A Remedial Action Plan covering both suspected and confirmed resistance for European corn borer, corn earworm, and southwestern corn borer is provided in the Enclosure. If resistance involves any of these three target pests, the registrant must

implement this Remedial Action Plan. The registrant must obtain approval from EPA before modifying the Remedial Action Plan for Lepidopteran-Protected Corn.

Annual Reports:

Beginning in the first year after commercialization, the registrant will provide annual reports to EPA on its moCry1F PIP expressed in corn based on the following table.

Report	Description	Due Date
Annual Sales	Reported by county and state summed by state	January 31st
Grower Agreement	Number of units of *Bt* corn seeds shipped or sold and not returned, and the number of such units that were sold to persons who have signed grower agreements	January 31st
Proposed Compliance Plan	Written description of Compliance Assurance Program	January 31s t
Compliance Assurance Plan	Compliance Assurance Program Results	January 31st
Compliance	To include annual survey results, changes to the education program, and plans for the next year	Preliminary survey report November 15th of the year of the survey and the full report the following January 31st
Insect Resistance Monitoring	Results of monitoring and investigations of damage reports.	April 30th

Prior to commercialization of moCry1F corn, additional reports are required as described in the following table:

IRM Grower Agreements	Proposed system to assure growers sign grower agreements
IRM Affirmation Plan	System to assure annual affirmation by growers of their IRM obligations
Changes to Grower	Grower agreement(s) and any specific stewardship documents.

Agreement and/or IRM documents	These are also required at least 30 days before any changes related to IRM are expected to be imposed.
Insect Resistance Monitoring Results	Description of the program including sampling (number of locations and samples per locations), sampling methodology, bioassay methodology, standardization procedures, detection technique and sensitivity, and the statistical analysis of the probability of detecting resistance.

IV. Regulatory Position

Pursuant to FIFRA section 3(c)(7)(C), EPA may conditionally register a new pesticide active ingredient if: 1) insufficient time has elapsed since the imposition of the data requirement for those data to be developed and all other required data have been submitted, 2) the use of the pesticide product during the period of the conditional registration will not cause any unreasonable adverse effect on the environment, and 3) the registration and use of the pesticide during the conditional registration is in the public interest. BPPD believes that all these criteria have been fulfilled.

The first criterion under FIFRA section 3(c)(7)(C) mentioned above has been met since insufficient time has elapsed since the imposition of the data requirements for:

1. The protocol for the Independent Lab Validation of analytical method GRM02.13 "Determination of Cry1F Insecticidal Crystal Protein in corn grain by Enzyme Linked Immunosorbant Assay" does not include a transgenic moCry1F treatment to substantiate the lack of interference posed by transgenic plant expression of Cry1F. Modify the protocol and submit to the EPA appropriate documentation to demonstrate that this method works to quantify Cry1F expressed in transgenic moCry1F corn on or before November 15, 2005.

2. Submit to the EPA laboratory (Ft. Meade, MD) methodology and/or reagents necessary for validation of a moCry1F analytical method within 6 months of the date of registration (under OPPTS Guidelines 860.1340). The extraction and detection method and independent third party laboratory validations as described for moCry1F protein appear to be adequate for analysis of moCry1F protein in corn grain. However, this method must be validated by both an independent laboratory and the EPA Biological and Economic Analysis Division laboratory before it can be

considered a valid method.

3. Submit confirmatory testing of moCry1F protein levels in soil under a range of conditions typical of *Bt* corn cultivation. EPA requires Mycogen Seeds c/o Dow AgroSciences LLC in cooperation with other registrants to submit test protocols before the studies are actually conducted. In general, the Agency anticipates that soils would be sampled from fields where *Bt* corn has been grown continuously for at least 3 years compared with fields where no *Bt* crop has been grown. These paired fields would include several locations throughout the corn growing area of the US representing different soil and climatic variations. The Agency anticipates that samples would need to be taken 2 or 3 times during the growing season. A protocol was approved for poCry1F in 2002. Submit a final report to the Agency for moCry1F by March 15, 2008.

4. Submit confirmatory field data for possible impacts of moCry1F on non-target insects. Preliminary data for poCry1F corn were provided to the Agency on March 14, 2002 and found to be acceptable. Submit a final report for moCry1F to the Agency by March 15, 2008.

The applicants have submitted or cited data to satisfy the second criterion for conditional registration under FIFRA 3(c)(7)(C) as mentioned above. Dow submitted and/or cited satisfactory data pertaining to the proposed use. The human health effects data and non-target organism effects data are considered sufficient for the period of the conditional registration. These data demonstrate that no foreseeable human health hazards or ecological effects are likely to arise from the use of the product and that the risk of resistance developing to *Bacillus thuringiensis* during the conditional registration is not expected to be significant. The data also demonstrate that there is virtually no possibility of any risk associated with weediness or outcrossing to wild relatives.

Registration of *Bacillus thuringiensis* subspecies Cry1F protein and the genetic material necessary for its production (plasmid insert PHI 8999) in Event TC1507 corn [plant-optimized Cry1F 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.

Registration of *Bacillus* thuringiensis subspecies Cry1F protein and the genetic material necessary for its production (plasmid insert PHP12537) [maize-optimized Cry1F corn] in Event TC6275 corn is in the public interest because it is, just as for plant-optimized Cry1F-protected corn, comparatively less risky to health or the environment than currently registered pesticides and the expected benefits (including economic benefits) from the use of the new active ingredient are greater than those of alternative registered pesticides and other available non-chemical techniques. Maize-optimized Cry1F-protected corn will solidify and extend the benefits of insecticide use reduction that have been

established for plant-optimized Cry1F-protected corn. The registered alternatives commonly used to treat the target pest complex protected by Cry1F are restricted use for the most part. They have precautionary label statements such as extremely toxic to fish and aquatic organisms, wildlife and require protective clothing for workers. The specific organophosphate and pyrethroid pesticides likely to be replaced are ranked in the top 15 of all pesticides with respect to reported incidents of mortality to non-target wildlife. Many of these products also control corn root worm, which is the most significant pest of corn and is frequently treated along with the target pest complex of Cry1F. Compared to other Bt corn products, growers are likely to choose Cry1F protected corn due to better product performance and broader spectrum of control. Cry1F protected corn is also expected to be economical on some unprotected fields and provide insurance against the risk of crop loss and the need to replant. But without root worm protection, the use of Cry1F to reduce conventional pesticide use is somewhat limited. Economic benefits to growers are the incremental improvement to grower profits compared to current practice.

In view of these minimal risks and the benefits, BPPD believes that the use of the product during the limited period of the conditional registration will not cause any unreasonable adverse effects.

Although the data with respect to this particular new active ingredient are satisfactory, it is not sufficient to support an unconditional registration under FIFRA 3(c)(5). Additional data are necessary to evaluate the risk posed by the continued use of this product. Consequently, data requirements specified earlier in Section III were required.

BPPD also believes, as explained in section II.E., that the third criterion for a FIFRA 3(c)(7)(C) conditional registration has been fulfilled because the use of Cry1F corn under this registration is in the public interest.

The related final tolerance rule for these registrations involves the plant-incorporated protectant Cry1F protein in corn and is found in 40 CFR 180.1217.

V. Actions Required by Registrants

IRM terms and conditions must be complied with, conditionally required data must be submitted, and reports of incidences of adverse effects to humans or domestic animals and target pest resistance must be submitted under FIFRA, Section 6(a)2.

Appendix 1

Remedial Action Plan for Responding to Resistance in European Corn Borer
Corn Earworm and/or Southwestern Corn Borer

I. Definitions

Suspected resistance

EPA defines "suspected" resistance to mean, in the case of reported product failure, that:

- the corn in question has been confirmed to be *Bt* corn
- the seed used had the proper percentage of corn expressing *Bt* protein;
- the relevant plant tissues are expressing the expected level of *Bt* protein; and
- it has been ruled out that species not susceptible to the protein could be responsible for the damage, that no climatic or cultural reasons could be responsible for the damage, and that other reasonable causes for the observed product failure have been ruled out.

The Agency does not interpret "suspected resistance" to mean grower reports of possible control failures, nor does the Agency intend that extensive field studies and testing to confirm scientifically insect resistance be completed before responsive measures are undertaken.

If resistance is "suspected," the registrant must instruct growers to do the following:

- Use alternate control measures to control the pest suspected of resistance to *Bt* corn in the affected region.
- Destroy crop residues in the affected region immediately after harvest (i.e. within one month) with a technique appropriate for local production practices to minimize the possibility of resistant insects overwintering and contributing to the next season's pest population.

Confirmed Resistance

The registrant assumes responsibility for the implementation of resistance mitigation actions undertaken in response to the occurrence of resistance during the growing season. When resistance has been confirmed, the registrant must immediately stop sale and distribution of *Bt* corn in the remedial action zone (may be less than a single county, single county, or multiple counties) where the resistance has been shown until an effective local mitigation plan approved by EPA has been implemented.

A resistance event becomes confirmed if the progeny of the sampled ECB, CEW, or SWCB population would exhibit all of the following characteristics in bioassays initiated with neonates:

1. If there is > 30% survival and > 25% leaf area damaged in a 5-day bioassay using Cry1Ab-positive or Cry1F-positive leaf tissue under controlled laboratory conditions.

2. If standardized laboratory bioassays using diagnostic doses for ECB (Marçon et al. 2000), SWCB (Trisyono and Chippendale 1999), or CEW/CBW (USDA/ARS/SIMRU, unpublished) demonstrate resistance has a genetic basis and survivorship in excess of 1% (gene frequency of population 0.1).

3. If an LC_{50} in a standard Cry1Ab or Cry1F diet bioassay exceeds the upper limit of the 95% confidence interval of the standard unselected laboratory population LC_{50} for susceptible ECB, SWCB, or CEW populations, as established by the ongoing baseline monitoring program.

II. Remedial Action

The registrant assumes responsibility for the implementation of resistance mitigation actions undertaken in response to the occurrence of resistance during the growing season. In cases of "confirmed" resistance, the following strategy for Cry1Ab and/or Cry1F *Bt* corn hybrids:

The registrant will report all instances of confirmed pest resistance, as defined above, to the Agency within 30 days. Upon identification of a confirmed instance of resistance, registrants will take the following immediate mitigation measures:

1. Notify customers and extension agents in the affected area,

2. Require to customers and extension agents in the affected area the use of alternative control measures to reduce or control the local target pest population,

3. Where appropriate, require to customers and extension agents in the affected area that crop residues be incorporated into the soil following harvest, to minimize the possibility of overwintering insects.

4. Immediately stop sale and distribution of *Bt* corn in the remedial action zone (may be a single or multiple counties) where the resistance has been shown until an effective local mitigation plan approved by EPA has been implemented.

Within 90 days of a confirmed instance of pest resistance, as defined above, registrants will:

1. Notify the Agency of the immediate mitigation measures that were implemented,

2. Submit to the Agency a proposed long-term resistance management action plan for the affected area,

3. Work closely with the Agency in assuring that an appropriate long-term resistance management action plan for the affected area is implemented, and

4. Implement an action plan that is approved by EPA and that consists of some or all the following elements, as warranted:

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

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

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

d. Implementing intensified local IRM measures in the affected area based on the latest research results. The implementation of such measures will be coordinated by the Agency with other registrants; and

e. The implementation of the remedial action strategy will be coordinated by the Agency with other registrants and stakeholders.

For mitigation of resistance in the growing season(s) following a confirmed resistance incident(s), use of the following procedures:

1. Maintenance of the sales suspension of all *Bt* corn hybrids (with the same protein or similar *Bt* proteins as the *Bt* corn hybrids with the resistant population) in the affected region would remain in place until an EPA-approved local resistance management plan is in place to mitigate resistance in the affected region(s).

2. The development and recommendation of alternative resistance management strategies for controlling the resistant pest(s) on corn in the affected region.

3. Notification of all relevant personnel (e.g., growers, consultants, extension agents, seed distributors, processors, university cooperators, and state/federal authorities) in the affected region of the resistance situation.