USDA/APHIS Decision on Dow AgroSciences and Pioneer Hi-Bred International Petition 03-353-01P Seeking a Determination of Nonregulated Status for Bt *cry34/35Ab1* Insect Resistant Corn Line DAS-59122-7

Environmental Assessment

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<u>APPENDICES</u>

I. SUMMARY

The Animal and Plant Health Inspection Service (APHIS), United States Department of Agriculture (USDA), has prepared an Environmental Assessment (EA) in response to a petition (APHIS Number 03-353-01p) from Dow AgroSciences and Pioneer Hi-Bred International (Dow/Pioneer) regarding the regulatory status of genetically engineered (transformed) corn rootworm resistant corn derived from their transformation event DAS-59122-7. This corn is currently a regulated article under USDA regulations at 7 CFR Part 340, and as such, interstate movements, importations, and field tests of DAS-59122-7 corn have been conducted under permits issued or notifications acknowledged by APHIS. Dow/Pioneer petitioned APHIS requesting a determination that DAS-59122-7 corn does not present a plant pest risk, and therefore DAS-59122-7 corn and its progeny derived from crosses with other nonregulated corn should no longer be regulated articles under these APHIS regulations.

The DAS-59122-7 corn has been genetically modified to express the c*ry34Ab1* and *cry35Ab1* genes from *Bacillus thuringiensis (Bt)* strain 149B1. These genes encode for a binary insecticidal crystal protein (formally known as PS149B1) composed of Bt Cry proteins Cry34Ab1 and Cry35Ab1 that protects the corn plants against the feeding damage of larvae of the corn rootworm (CRW) complex comprised of *Diabrotica* species. Insecticidal proteins have previously been engineered into corn to control pests in the insect order Lepidoptera as well as to the order Coleoptera, the order to which *Diabrotica* species belong. As with the previous Bt Cry proteins, Cry34Ab1 and Cry35Ab1 have a high degree of specificity for the target pest. DAS-59122-7 corn also expresses the *pat* gene which encodes for the enzyme phosphinothricin acetyltransferase (PAT) and confers tolerance to glufosinate-ammonium herbicides. The presence of the PAT gene in DAS-59122-7 provides an alternative weed management tool to growers and a method for selecting for corn that contains the transgenes.

Field trials with DAS-59122-7 corn have been conducted under the APHIS notification procedure (7 CFR Part 340.3). Performance standards for such field trials require that the regulated article and its offspring must not persist in the environment after completion of the test. In accordance with APHIS procedures for implementing the National Environmental Policy Act (NEPA) (7 CFR Part 372), this EA has been prepared prior to issuing a determination of nonregulated status for DAS-59122-7 corn in order to specifically address the potential for impact to the human environment through the unconfined cultivation and use in agriculture of the regulated article.

II. BACKGROUND

A. Development of DAS-59122-7 corn

Dow/Pioneer has submitted a "Petition for Determination of Non-regulated Status" to the USDA/APHIS (APHIS number 03-353-01p) for genetically engineered corn plants that are resistant to the feeding damage caused by: the northern corn rootworm (nCRW, *D. barberi*); the western corn rootworm (wCRW, *Diabrotica virgifera virgifera*); and the Mexican corn rootworm (mCRW, *D. virgifera zeae*). The corn rootworm larvae damage corn by feeding on the roots of corn plants, thereby inhibiting the ability of the plant to absorb water and nutrients from the soil (Reidell, 1990). This leads to harvesting difficulties due to lodging of the weakened plants (Spike and Tollefson, 1991). Annual losses to growers because of CRW have been estimated to approach a billion dollars when taking into account both the costs of chemical controls and crop losses from CRW (USDA-ARS, 2003). Dow/Pioneer has submitted data indicating economically significant levels of control against the corn rootworm pests, wCRW, nCRW, and mCRW.

Bacillus thuringiensis bacteria produce a group of related toxins (delta-endotoxins) that when ingested by susceptible insects (such as coleopterans and lepidopterans) result in insect death. Preparations of Bt containing delta-endotoxins have been used for decades as foliarly applied biopesticides. However, these foliar applications are not routinely effective against CRW pests because the insect pests reside in the soil. Similar problems can be encountered with other, non-systemic, foliarly applied chemical insecticides. The development and approval of transgenic corn plants expressing Bt delta-endotoxins active against coleopterans (e.g., Cry34Ab1 and Cry35Ab1) should provide growers with another safe and efficacious option for the control of CRW.

Dow/Pioneer used recombinant DNA techniques to produce and introduce into corn, a restriction fragment containing the *cry34Ab1*,*cry35Ab1*, and *pat* genes from *Bacillus thuringiensis* strain PS149B1 and *Streptomyces viridochromogenes*, respectively. Expression of these genes by corn plants renders the corn line resistant to CRW and tolerant to the herbicide glufosinate. Regulatory elements for the *cry34Ab1*, *cry35Ab1*, and *pat* genes were derived from the plant pathogenic virus cauliflower mosaic virus (CaMV), potato, and corn. These regulatory sequences are not transcribed and do not encode proteins. The DNA was introduced into corn cells using Agrobacterium-mediated transformation methodology with the T-DNA transformation vector designated PHP 17662. In addition to transgenes necessary for insertion into the plant genome, the T-DNA vector also contained within the backbone two genes conferring bacterial resistance to the antibiotics spectinomycin and tetracycline, and the bacterial origin of replication. The recipient corn line used in the transformation was the public line designated Hi-II. Plant cells containing the introduced DNA were then selected by culturing in the presence of glufosinate-ammonium. In addition, antibiotics included in the culture medium killed any remaining Agrobacterium. Because the transformed cells contain some sequences from plant pathogens, they are explicitly subject to regulation under 7 CFR Part 340.

DAS 59122-7 corn has been field tested in the United States since 2001 as authorized by USDA notifications and permits listed in Appendix A. The list compiles a number of test sites in diverse regions of the U.S. including the major corn growing areas of the Midwest and winter nurseries in Hawaii and Puerto Rico. Field tests conducted under APHIS oversight allow for evaluation in a natural agricultural setting while imposing measures to minimize the risk of persistence in the environment after the completion of the test. Data are gathered on multiple parameters and are used by the applicants to evaluate agronomic characteristics and product performance and are used by APHIS to determine if the new variety poses a plant pest risk.

B. APHIS Regulatory Authority.

APHIS regulations under 7 CFR Part 340, which are promulgated pursuant to authority granted by the Plant Protection Act (Title IV, Pub. L. 106-224, 114 Stat. 438, 7 U.S.C. 7701-7772) regulate the introduction (importation, interstate movement, or release into the environment) of certain genetically engineered organisms and products. A genetically engineered organism is considered a regulated article if the donor organism, recipient organism, vector or vector agent used in engineering the organism belongs to one of the taxa listed in the regulation and is also a plant pest, or if there is reason to believe that it is a plant pest. DAS-59122-7 corn has been considered a regulated article because some non-coding DNA regulatory sequences were derived from plant pathogens.

Section 340.6 of the regulations, entitled "Petition for Determination of Nonregulated Status", provides that a person may petition the Agency to evaluate submitted data and determine that a particular regulated article does not present a plant pest risk and should no longer be regulated. If APHIS determines that the regulated article is unlikely to pose a greater plant pest risk than the unmodified organism from which it is derived, the Agency can grant the petition in whole or in part. Therefore, APHIS permits or notifications would no longer be required for field testing, importation, or interstate movement of that article or its progeny.

C. U.S. Environmental Protection Agency (EPA) and Food and Drug Administration (FDA) Regulatory Authority.

DAS-59122-7 corn is also subject to regulation by other agencies. The EPA is responsible for the regulation of pesticides under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136 *et seq.*). FIFRA requires that all pesticides, including herbicides, be registered before distribution or sale, unless exempt by EPA regulation. Before a product may be registered as a pesticide under FIFRA, it must be shown that when used in accordance with widespread and commonly recognized practices, it will not cause unreasonable adverse effects on the environment. On March 13, 2003, the EPA announced the receipt of an application filed by Dow/Pioneer for an Experimental Use Permit (EUP) for field testing of the Cry 34Ab1 and Cry35 Ab1 proteins and the genetic material necessary for their production in corn (68 *FR* 12073-12076, March 13, 2003). Subsequently, Dow/Pioneer applied for an extension to the EUP that expires April 30, 2006 (69 *FR* 11431-11433). On September 1, 2004, the EPA announced the receipt of an application filed by Dow/Pioneer to register this new pesticidal product and the genetic material necessary of its production in corn (69 *FR* 53434-53436). These active ingredients are not included in any previously registered products.

Under the Federal Food, Drug, and Cosmetic Act (FFDCA) (21 U.S.C. 301 *et seq.*), pesticides added to (or contained in) raw agricultural commodities generally are considered to be unsafe unless a tolerance or exemption from tolerance has been established. Residue tolerances for pesticides are established by EPA under the FFDCA. The FDA enforces the tolerances set by the EPA. On July 7, 2003, EPA granted a temporary tolerance exemption for Cry34Ab1 and Cry35Ab1 (68 *FR* 40178-40183, July 7, 2003). The temporary tolerance, in effect through April 30, 2006, concluded that there was a reasonable certainty of no harm from consumption of the protein, as it is digestible in gastric fluid and not considered an allergen. On August 31, 2004, the

EPA announced the receipt of a petition to establish an exemption from the requirement of a tolerance for the Cry34Ab1 and Cry 35Ab1 proteins found in food (69 *FR* 53060-53063).

FDA's policy statement concerning regulation of products derived from new plant varieties, including those genetically engineered, was published in the Federal Register on May 29, 1992, and appears at 57 *FR* 22984-23005. Under this policy, FDA uses what is termed a consultation process to ensure that human food and animal feed safety issues or other regulatory issues (e.g. labeling) are resolved prior to commercial distribution of a bioengineered food. Dow/Pioneer submitted on a summary of their safety assessment on December 11, 2003, and additional information on March 5 & 12, 2004. The Dow/Pioneer assessment to the FDA indicated no changes in composition, safety or other relative parameters. The consultation for DAS-59122-7 corn as food and feed was completed on October 4, 2004 indicating that the FDA has no unresolved issues with respect to the use of DAS-59122-7 for human food and animal feed.

III. PURPOSE AND NEED

In compliance with the National Environmental Policy Act (NEPA) of 1969 (42 U.S.C. 4321 *et seq*.) and the pursuant implementing regulations (40 CFR 1500-1508; 7 CFR Part 1b; 7 CFR Part 372), APHIS has prepared this EA before making a determination on the status of DAS-59122-7 corn as a regulated article under APHIS regulations. The developer of DAS-59122-7 corn, Dow/Pioneer, submitted a petition requesting that APHIS make a determination that corn transformation event DAS-59122-7, and any progeny derived from crosses of event DAS-59122-7 with other nonregulated corn varieties, no longer be considered regulated articles under 7 CFR Part 340.

IV. ALTERNATIVES

A. No Action: Continuation as a Regulated Article

Under the no action alternative, APHIS would come to a determination that DAS-59122-7 corn and its progeny should continue to be regulated under 7 CFR Part 340. Permits or acknowledgment of notifications from APHIS would still be required for their introduction. APHIS would choose this alternative if there were insufficient evidence to demonstrate lack of plant pest risk from the uncontained cultivation of DAS-59122-7 corn and its progeny.

B. Determination of Nonregulated Status

Under this alternative, DAS-59122-7 corn and its progeny would no longer be considered regulated articles under 7 CFR Part 340. Permits or notifications to APHIS would no longer be required for introductions in the United States and its territories of DAS-59122-7 corn or its progeny. A basis for this determination would be a finding that DAS-59122-7 is unlikely to pose a greater plant pest risk than the non-modified organism from which it was derived based on information submitted in the petition as stipulated in 7 CFR Part 340.6 (c) and other information that the Administrator believes to be relevant to a determination. Unrestricted cultivation of the lines would be permitted by APHIS. Such a determination, however, does not preclude any restriction on the cultivation of this corn that might be placed by other regulatory agencies also having authority.

C. Determination of Nonregulated Status, in Part

The regulations at 7 CFR Part 340.6 (d) (3) (i) state that APHIS may Aapprove the petition in whole or in part.@ There are two ways in which a petition might be approved in part:

Approval of some but not all of lines requested in the petition. In some petitions, applicants request de-regulation of lines derived from more that one independent transformation event. In these cases, supporting data must be supplied for each line. APHIS could approve certain lines requested in the petition, but not others.

Approval of the petition with geographic restrictions. APHIS might determine that the regulated article poses no significant risk in certain geographic areas, but may pose a significant risk in others. In this case, APHIS may choose to approve the petition with a geographic limitation stipulating that the approved lines could only be grown in certain geographic areas based on the identification of site-specific risks.

V. POTENTIAL ENVIRONMENTAL IMPACTS

The potential environmental impacts of alternatives A and B, as described above in section IV are presented in this section.

Alternative A, Non Action.
In a decision to choose alternative A., no action, DAS-59122-7 corn plants would still require APHIS authorization to be planted. In this case measures would need to continue to be implemented to ensure physical and reproductive confinement of corn DAS-59122-7 and any progeny derived from it.

If growers do not have improved varieties of corn seed derived from corn line DAS-59122-7, they may choose to plant another cultivar with similar properties as an alternative, or they may use other chemical or biological control mechanisms or management practices if they feel that their coleopteran pest pressure and weed pressure is high enough to warrant it.

Another deregulated transgenic coleopteran resistant corn expressing Bt Cry3Bb1 delta-endotoxin (YieldGard®) was deregulated in 2002. YieldGard® was planted on about 0.5% of total corn acreage in 2003, limited by seed supply (Sankula and Blumenthal, 2004). Adoption of this CRW resistant corn line is expected to continue to increase in the coming years as more seed becomes available to growers (Sankula and Blumenthal, 2004). Other herbicide tolerant corn varieties are also available from seed companies, and have been widely adopted by farmers in the United States (Sankula and Blumenthal, 2004; Fernandes-Cornejo and McBride, 2000; Carpenter and Gianessi, 1999). Herbicide tolerant varieties include the transgenic Liberty Link® varieties resistant to the herbicidal active ingredient glufosinate-ammonium (e.g. as found in the herbicide

Liberty ® which is registered in the United States for use on seed designated as Liberty Link®), transgenic Roundup Ready® varieties resistant to the herbicidal active ingredient glyphosate (as found in the herbicide Roundup®), as well as non-transgenic varieties resistant to two other types of herbicides: the acetolactate synthase (ALS) inhibiting herbicide imidazolinone (IMI) and sethoxydim (Knake, 1998; Fernandez-Cornejo and McBride, 2000). Several chemical insecticides and biological or cultural control measures can be used to control the pests targeted by Bt DAS-59122-7 corn, and several herbicides and cultural practices can be used to manage weeds in corn. However, there are no corn products currently available that combine both CRW resistance and glufosinate-ammonium tolerance.

Corn rootworms are the most serious insect pests in field corn in the U.S., costing growers millions of dollars each year in terms of insecticide use and crop loss (Sankula and Blumenthal, 2004). Historically, crop rotation has provided effective protection from CRW damage. More recently, however, the effectiveness of crop rotation has become more limited because of several factors:

1. Many growers now prefer to grow corn continuously, as opposed to using crop rotation. Continuous corn production is a practice that necessitates higher inputs of chemical insecticides. The percentage of continuous corn acreage in the eastern and western Corn Belt states treated with insecticides ranges from 7%-100% (Gianessi, et al., 2002).

2. Crop rotation is not an effective management strategy for southern corn rootworm (sCRW) because it not only has a wide host range, but also because multiple generations can be produced in the same corn field (Gianessi, et al., 2002). Larvae of sCRW can be found on the roots of corn, peanuts, alfalfa and cucurbits. There may be two to three generations of sCRW per year. Adults become active and lay eggs in the soil in late spring. These eggs hatch after one week and the larvae feed on corn roots for two to four weeks before pupating. A new generation of adults can emerge in mid-summer (Gianessi, et al., 2002).

3. A new nCRW biotype has exhibited extended diapauses in which some eggs can survive through a non-corn rotation to attack corn in a subsequent season (Ostlie, 1987; Tollefson, 1988; Gray *et al.*, 1998; Gianessi, 2002). In South Dakota, Minnesota, Iowa, and Nebraska, the new nCRW biotype can diapause for two winters which allows the eggs to bypass the rotated crop and hatch in time to feed on the next corn crop (Gianessi, 2002).

4. A new biotype of wCRW has appeared in central Illinois, northern Indiana and parts of Michigan that can lay eggs in soybean fields, so that the eggs hatch in the following season coinciding with the corn rotation (Onstad and Joselyn, 1999; O'Neal et al., 1999; Gianessi, 2002). This strain has spread rapidly since it was first observed in 1993, and it is expected to continue to spread throughout the Corn Belt.

As a result of these factors and the very damaging nature of the pest, the CRW complex is the most significant corn pest in the U.S. in terms of organophosphate chemical pesticide usage. The most common chemical regime is the application of a granular insecticide at planting, either banded or in-furrow. In some cases sprays are applied for adult suppression. Widespread use of chemical insecticides has raised concerns for worker safety, water contamination, and other environmental risks. Appendix C is a table in which some of the most commonly used chemicals are compared with respect to environmental fate and toxicity.

If growers do not have improved varieties of corn seed derived from line DAS-59122-7, they may choose to plant another cultivar with similar properties as an alternative, or they may use other chemical or biological control mechanisms or management practices. APHIS envisions no significant adverse impacts over and above those associated with current practices.

Alternative B, Determination of Nonregulated Status.
A decision to choose alternative B, deregulation of DAS-59122-7 corn, is addressed below. The unrestricted cultivation and distribution of DAS-59122-7 corn is compared to that for other corn not subject to regulation by APHIS under 7 CFR Part 340.

A. Plant Pathogenic Properties

APHIS considered the potential for the transformation process, the introduced DNA sequences, or their expression products to cause or aggravate disease symptoms in DAS-59122-7 corn or other plants or to cause the production of plant pathogens. We also considered whether data indicate that unanticipated plant pest effects would arise from cultivation of DAS-59122-7 corn.

Line DAS-59122-7 corn was generated using plasmid PHP 17662 (described in Figure 1 of Petition) via *Agrobacterium*-mediated transformation. Transformation with *Agrobacterium* should not lead to crown gall disease in DAS-59122-7 because the *Agrobacterium tumefaciens* strain LBA4404 was disarmed by removing the native T-DNA from LBA4404. The native T-DNA contains the plant hormone genes necessary for the formation of crown gall tumors. Instead, a T-DNA region that contains synthetic, maize-optimized *cry 34Ab1* and *cry35Ab1* genes, as well as the synthetic *pat* gene coding sequences and the regulatory components necessary for their expression in the corn genome, was introduced on a binary plasmid to create plasmid PHP 17662 (described in Figure 2 of petition). Further, antibiotics were used to kill any remaining *Agrobacterium* after the transformation. For the transformation process, plant cells from the publicly available inbred corn line Hi-II were used as the recipient material. Hi-II is a derivative of the A188 and B73 inbred lines which are publicly available inbred lines form the University of Minnesota and Iowa State University, respectively.

APHIS analyzed data that demonstrates that DAS-59122-7 corn plants regenerated from the transformation event designated DAS-59122-7 contains one copy of the following genetic elements from the plasmid PHP 17662 (see Petition Table 3): (1) the coding sequences of the synthetic *cry 34Ab1* and *cry35Ab1* insecticidal protein genes from *Bacillus thuringiensis* strain 149B1 whose transcription is directed by the maize ubiquitin promoter (UBI1ZM PRO) and wheat (*Triticum aestivum)* peroxidase (TA Peroxidase) promoters respectively. The termination sequences for these two genes were derived from the potato (*Solanum tuberosum)* proteinase inhibitor II (PINII); and (2) the coding sequence of the synthetic *pat* gene derived from the bacterium *Streptomyces viridochromogenes* that encodes the enzyme phosphinothricin acetyltransferase (PAT) the transcription of which is driven by the cauliflower mosaic virus (CaMV) 35S RNA promoter and with a CaMV termination sequence (CaMV35S TERM).

The donor organisms for the *cry34Ab1*, *cry35Ab1* and *pat* genes (*Bt* strain PS149B1 and *Streptomyces viridochromogenes*, respectively) are soil-inhabiting bacteria. Neither of these bacteria are plant or human pathogens, and the Cry34Ab1, Cry35Ab1 and PAT proteins encoded by these genes do not cause disease symptoms or the production of infectious agents in plants. The synthetic, maize-optimized *cry34Ab1* and *cry35Ab1* coding sequences were modified for optimal expression in corn, in part, by changing their codon bias to that favored by corn. The synthetic, plant optimized *pat* coding sequence provides for improved expression of PAT in corn plants. The promoter for the *pat* gene is the 35S promoter derived from cauliflower mosaic virus which is a plant pathogen. Cauliflower mosaic virus causes disease primarily in cruciferous plants. However, the CaMV 35S promoter does not cause disease symptoms in plants, nor does it encode for an infectious agent.

Seeds obtained from two breeding generations (T1S1 and BC1) were used for DNA analysis of event DAS-59122-7. The T1S1 generation represented the original Hi-II corn line containing event DAS-59122-7 (T0) crossed to the elite inbred PH09B to produce an F1 hybrid (T1), and then selfed to give T1S1 seed. The BC1 generation represented the first backcross generation of the T1 event DAS-59122-7 with the recurrent parent 05F. The BC1 generation was then crossed to a second inbred (581) to produce the hybrid seed. Plants from both generations were grown in growth chambers and leaf samples from test and unmodified control plants (Hi-II, P38 – inbred control, PH09B – inbred control, 05Fx581 – hybrid control) that contain a genetic background representative of the transgenic plants were obtained for DNA extraction and analysis. Southern blot analysis of these samples was performed using probes for the *cry34Ab1, cry35Ab1,* and *pat* genes. Descriptions of the DNA probes used for Southern hybridization are found in Table 4, page 30 of the Petition.

Seeds were also obtained from two plant breeding generations of event DAS-59122-7 (BC2S1 and T1S2). The BC2S1 seed represents transformation into a Hi-II background followed by an outcross for one generation to inbred line PH09B with the resulting F1 crossed and then backcrossed twice to inbred 581 to make a BC2. The BC2 generation was self-crossed to generate the segregating population. The T1S2 generation represented event 59122-7 in Hi-II (T0) crossed to elite inbred PH09B to give an F1 hybrid (T1), and then selfed twice to give T1S2 seed. Seeds were again grown in growth chambers and leaf samples were obtained for analysis. Southern blot analysis indicated that a single copy of the intact T-DNA region from plasmid PHP 17662 was inserted into the corn genome (Table 6 & 7, page 47-48 of Petition).

Figure 51 of the Petition (Page 101) provides a schematic diagram of the Mendelian inheritance of Cry34/35Ab1 corn. Mendelian segregation of the DAS-59122-7 corn event was analyzed using Chi-square analysis at eight generations (Table 14, Page 100 of Petition). Each generation of plants were treated with glufosinate-ammonium to eliminate those plants that were not herbicide-tolerant, reflecting a lack of inheritance of the *pat* gene. Each plant that was herbicide-tolerant was subsequently tested for the presence of the Cry34Ab1 protein by immunoassay.

Each plant that was herbicide-tolerant, also was positive for the presence of the Cry34Ab1 protein as expected. Table 14 of the Petition shows the expected and observed segregation for the eight generations. In five of the eight generations tested, there was no significant deviation from the expected 3:1 segregation ratio. However, in three of the five generations (BC1, BC2, and BC4S1), there was significant deviation from the expected segregation ratio. Dow/Pioneer discussed two possibilities for this deviation. Since the segregation ratio deviation only occurred in one of the two inbreds from each generation, Dow/Pioneer suggests that one possible explanation is a relatively small sample size for the BC1 generation and that with a larger sample size, the segregation ratios would more likely reflect the expected 3:1 ratio. A second potential explanation provided by Dow/Pioneer is that a breeding error may have occurred that allowed extra susceptible plants in the BC1, BC4 and BC4S1 generations. Despite the deviation seen in these three generations, when the Chi-square test was performed across all generations, the expected 3:1 segregation ratio is obtained, consistent with a dominant trait at a single locus for corn event DAS-59122-7.

Additional DNA analysis using other DNA restriction fragments were performed to support the conclusion that corn line DAS-59122-7 contains within its genomic DNA (nuclear chromosomes) a single copy of an insertion containing the complete coding regions for the *cry34Ab1*, *cry35Ab1*, and *pat* genes with the associated noncoding regulatory regions (see Petition, Section V.A., Table 6 & 7, Figures 4-28; Appendix 2, Section 6-7; Tables 5-7).

To confirm the absence of the *virG*, *tet* and *spc* genes, as well as plasmid backbone, Southern blot analysis of the corn leaf tissue was performed. DNA samples from DAS-59122-7 corn, T1S1 and BC1 generations, as well as unmodified controls were digested (*Hin*d III, *Sac* I, and *Xho* I) and probed with one or more of the five backbone probes: *spc, tet, virG,* LB Backbone and RB Backbone. Positive controls consisted of control corn DNA spiked with PHP 17662 plasmid DNA digested with the same enzymes as described above. Negative controls consisted of DNA from unmodified corn samples. As expected, no hybridization signals were observed in the DAS-59122-7 corn samples, the null segregants nor the unmodified controls. The positive control samples produced the expected hybridization patterns. The results of these tests suggest the absence of the *spc, tet, virG* genes and plasmid backbone in corn event DAS-59122-7. Table 8 (Petition page 67) describes the predicted and observed hybridization bands from corn event DAS-59122-7 for this analysis. Figures 29-36 (Petition pages 68-75) correspond to the results presented in Table 8.

B. Potential impacts based on the relative weediness of MON 863 corn.

APHIS assessed whether DAS-59122-7 corn is any more likely to become a weed than the nontransgenic recipient corn line, or other corn currently cultivated. The assessment encompasses a thorough consideration of the basic biology of corn and an evaluation of unique characteristics of DAS-59122-7 corn.

In the United States, corn is not listed as a weed in the major weed references (Crockett, 1977; Holm et al., 1979; Muenscher, 1980), nor is it present on the lists of noxious weed species distributed by the Federal Government (7 CFR Part 360). Furthermore, corn has been grown throughout the world without any report that it is a serious weed. Cultivated corn is unlikely to become a weed. It is not generally persistent in undisturbed environments without human intervention. Although corn volunteers are not uncommon, they are easily controlled by herbicides or mechanical means. Corn also possesses few of the characteristics of plants that are notably successful weeds (Baker, 1965; Keeler, 1989).

Dow and Pioneer conducted separate agronomic field trials at a total of 18 unique locations in the U.S. Corn Belt during the 2003 growing season. Field trial data (Tables 35-36 & 38-40) indicated that DAS-59122-7 corn does not exhibit characteristics that would cause it to be more weedy than the parental corn line. At these 18 locations, the range of values for agronomic parameters was within the range of values expected for traditional maize hybrids. In addition, data showed no significant differences between line DAS-59122-7 corn and the non-transgenic counterparts for disease and pest susceptibility (other than resistance to the targeted coleopteran pests). Traits evaluated include: yield (bushels/acre); percent moisture; accumulated maize growing degree days to reach 50% silking; grain density (lbs at 15.5% moisture); plant height (inches); ear height (inches); final stand count (average number of plants/acre in thousands); stalk lodging (percent of plants per plot showing lodging); root lodging (percent of plants per plot showing lodging); dropped ears per plot; top integrity (1-9 visual scale that describes how well stalks remain intact above ear).

The introduced traits, coleopteran insect resistance and glufosinate ammonium herbicide tolerance, are not expected to cause DAS-59122-7 corn to become a weed. Other CRW-resistant or glufosinate tolerant corn varieties previously deregulated by APHIS did not exhibit characteristics that would enhance weediness (APHIS assessments are available at: http://www.aphis.usda.gov/brs/not_reg.html). None of the characteristics of weeds described by Baker involve resistance or susceptibility to insects, and there is no reason to expect that the protection against the target insects provided by this new corn line would release it from any constraint that would result in increased weediness. DAS-59122-7 corn is still susceptible to other insect pests and diseases of corn and it is unchanged in its susceptibility to injury by commercially available herbicides other than glufosinate ammonium.

C. Potential impacts from gene introgression from DAS-59122-7 corn into its sexually-compatible relatives.

APHIS evaluated the potential for gene introgression to occur from DAS-59122-7 corn to sexually compatible wild relatives and considered whether such introgression would result in increased weediness. Cultivated corn, or maize, *Zea mays* L. subsp. *mays*, is sexually compatible with other members of the genus *Zea*, and to a much lesser degree with members of the genus *Tripsacum*.

Wild diploid and tetraploid members of *Zea* collectively referred to as teosinte are normally confined to the tropical and subtropical regions of Mexico, Guatemala, and Nicaragua; however, a fairly rare, sparsely dispersed feral population of teosinte has been reported in Florida. The Mexican and Central America teosinte populations primarily exist within and around cultivated maize fields; they are partially dependent on agricultural niches or open habitats, and in some cases are grazed upon or fed to cattle which distribute the seed. While some teosinte may be considered to be weeds in certain instances, they are also used by some farmers for breeding improved maize (Sánchez and Ruiz, 1997, and references therein). Teosinte is described to be susceptible to many of the same pests and diseases which attack cultivated corn (Sánchez and Ruiz, 1997).

All teosinte members can be crossed with cultivated corn to produce fertile F_1 hybrids (Doebley, 1990a; Wilkes, 1967). In areas of Mexico and Guatemala where teosinte and corn coexist, they have been reported to produce hybrids. Of the annual teosintes, *Z. mays* subsp. *mexicana* forms frequent hybrids with maize, *Z. luxurians* hybridizes only rarely with maize, whereas populations of *Z. mays* subsp. *parviglumis* are variable in this regard (Wilkes, 1977; Doebley, 1990a). Research on sympatric populations of maize and teosinte suggests introgression has occurred in the past, in particular from maize to *Z. mays* subsp. *luxurians* and *Z. mays* subsp. *diploperennis* and from annual Mexican plateau teosinte (*Z. mays* subsp. *mexicana*) to maize (Kato Y., 1997 and references therein).

Nonetheless, in the wild, introgressive hybridization from maize to teosinte is currently limited, in part, by several factors including distribution, differing degrees of genetic incompatibility, differences in flowering time in some cases, block inheritance, developmental morphology and timing of the reproductive structures, dissemination, and dormancy (Doebley, 1990a and 1990b; Galinat, 1988). First-generation hybrids are generally less fit for survival and dissemination in the wild, and show substantially reduced reproductive capacity which acts as a significant constraint on introgression. Teosinte has coexisted and co-evolved in close proximity to maize in the Americas over thousands of years, but maize and teosinte maintain distinct genetic constitutions despite sporadic introgression (Doebley, 1990a). The potential for gene introgression from DAS-59122-7 corn into teosinte would increase if varieties are developed, and approved for cultivation in locations where these teosintes are located. A limited potential can also occur through smuggling unapproved seeds or from import grain for planting. Since DAS-59122-7 corn does not exhibit characteristics that cause it to be any more weedy than other cultivated corn, its potential impact due to the limited potential for gene introgression into teosinte is not expected to be any different from that of other cultivated maize varieties.

The genus *Tripsacum* contains up to 16 recognized species, most of which are native to Mexico, Central and South America, but three of which exist as wild and/or cultivated species in the U.S. Though many of these species occur where corn might be cultivated, gene introgression from DAS-59122-7 corn under natural conditions is highly unlikely or impossible. Hybrids of *Tripsacum* species with *Zea* are difficult to obtain outside of a laboratory and are often sterile or have greatly reduced fertility, and none are able to withstand even the mildest winters. Furthermore, none of the sexually compatible relatives of corn in the U.S. are considered to be weeds in the U.S. (Holm et al., 1979), therefore, the unlikely acquisition of a single pesticide gene and an herbicide tolerance gene would not be expected to transform them into weeds.

D. Potential impact on nontarget organisms, including beneficial organisms and threatened or endangered species.

APHIS evaluated the potential for line DAS-59122-7 corn plants and their products to have damaging or toxic effects directly or indirectly on non-target organisms. Non-target organisms considered were those representative of the exposed agricultural environment, including those that are recognized as beneficial to agriculture or as threatened or endangered in the United States. APHIS also considered potential impacts on other "non-target" pests, since such impacts could potentially change agricultural practices.

The *pat* gene comes from *S. viridochoromogenes* and encodes the enzyme phosphinothricin acetyltransferase. *Pat* serves as a marker gene that enables selection of Bt lines and provides resistance to glufosinate-ammonium herbicides. The expression of PAT protein in corn plants is not expected to have deleterious effects or significant impacts on non-target organisms, including beneficial organisms, based on data provided in the petition and APHIS analyses of previously deregulated transgenic corn lines that express PAT proteins. The DNA encoding the PAT protein is not toxic and the PAT protein shares no significant homology with proteins known to be toxic or allergenic (OECD, 1999).

Like the Cry1 class of insecticidal proteins, the specificity of the Cry34Ab1 and Cry35Ab1 protein insecticidal activity is dependent upon their binding to specific receptors present in the insect mid-gut (Lambert, et al., 1996; Van Rie et al., 1990; Van Rie et al., 1989; Hoffmann et al., 1988a and 1988b; and Wolfersberger et al., 1986).

These insecticidal proteins are not expected to adversely affect other invertebrates or vertebrate organisms, including non-target birds, mammals and humans. APHIS evaluated laboratory and field studies on representative species that support these expectations. The toxicity and specificity of the Lepidopteran specific Cry proteins is associated with their solubilization and proteolytic activation in the insect midgut, and their binding to specific cell membrane receptors in the brush border membrane vesicles present in the midgut of susceptible insects. These specific receptors would not be expected to be present in non-target birds, mammals, and humans. (Griffitts, et al., 2005; Lambert, et al., 1996; Van Rie et al., 1990; Van Rie et al., 1989; Hoffmann et al., 1988a and 1988b; and Wolfersberger et al., 1986).

Potential impacts on non-target, non-lepidopteran pests:
The Cry34/35Ab1 binary protein only has activity against select beetle (Order: Coleoptera) species within the family Chrysomelidae, namely corn rootworm (CRW; *Diabrotica* subsp.). Dow AgroSciences conducted a series of diet bioassays with microbially-expressed Cry34Ab1 and Cry35Ab1 proteins to characterize the insecticidal specificity (see Petition Section V.F.1, Table 28). Test species were: northern corn rootworm (*Diabrotica barberi*), western corn rootworm (*Diabrotica virgifera virgifera*), southern corn rootworm (*Diabrotica undecimpunctata howardi*), European corn borer (*Ostrinia nubilalis*), corn earworm (*Helicoverpa zea*), black cutworm (*Agrotis ipsilon*) and corn leaf aphid (*Rhopalosiphum maidis*). Only corn rootworm (*Diabrotica* spp.) experience high mortality when exposed to microbially-expressed Cry34 and Cry35 proteins. Field trials of DAS-59122-7 corn plants also verified that Cry34/35Ab1 expressed in corn line DAS-59122-7 is active against wCRW (see Petition Section V.F.1, Table 29) and nCRW (see Petition Section V.F.1, Tables 30 and 31) but not active against non-target

lepidopterans such as the black cutworm (see Petition Section V.F.1, Table 32) and European corn borer (see Petition Section V.F.1, Table 33).

Potential impacts on non-target organisms, including beneficial organisms:
The Cry34Ab1 and Cry35Ab1 proteins are not expected to adversely affect non-target invertebrates and vertebrate organisms, including non-target birds, mammals and humans, because they would not be expected to contain the receptor protein found in the midgut of target insects. To evaluate the potential of line DAS-59122-7 corn to have damaging or toxic effects on representative terrestrial and an aquatic species, APHIS evaluated data from a series of ecological toxicology experiments. APHIS evaluated the results of several studies submitted that were designed to evaluate the sensitivity of representative non-target organisms to Cry34Ab1 and Cry35Ab1 proteins. Test substrates included corn plant material (e.g., corn grain, leaf or pollen) expressing modified Cry34Ab1 and 35Ab1 proteins or protein purified from a *Pseudomonas fluorescens* bacterial strain engineered to express the Cry34/35Ab1 binary protein. Dow AgroSciences verified that the bacterially-produced Cry34Ab1 and Cry35Ab1 proteins, as purified and prepared for these studies, were similar enough in its biochemical properties (molecular weight, amino acid sequence, and lack of glycosylation) and in biological activity against chrysomelids to warrant use as a test substance comparable to Cry34/35Ab1 as produced in line DAS-59122-7 corn.

Acute dietary toxicity studies of beneficial arthropods were conducted in laboratory tests and no adverse effects were observed at levels 10 to 100 times the expected field exposure (high end exposure estimates – HEEE). Honey bee (*Apis mellifera*) survival and development was tested as a pollinating species with diets including pollen expressing the Cry34/35Ab1 binary protein as well as microbially-produced protein. Beneficial natural enemies including green lacewings (*Chrysoperla carnea*), a parasitic Hymenoptera (*Nasonia vitripennis*) and lady beetles (*Hippodamia convergens* and *Coleomegilla maculata*) were fed microbially-produced protein. Representative decomposers including Collembola (*Folsomia candida*) and earthworms were also evaluated using microbially-produced Cry34Ab1 and Cry35Ab1 proteins. Other non-target organism tests included a freshwater invertebrate (*Daphnia magna*) and rainbow trout fed microbially-produced protein and chicken broilers fed grain from event DAS-59122-7. All of the organisms evaluated in the dietary toxicity studies were exposed to much greater levels of the Cry34 and Cry35 proteins than they would be exposed in the field (see Petition Section VI.B., Table 41) with no adverse effects observed.

Since parasitic and predatory insects will have limited direct exposure to the Cry34Ab1 and Cry35Ab1 insecticidal proteins expressed in line DAS-59122-7 corn, little impact is expected for these species other than a possible shift to alternate hosts since corn rootworm populations are expected to be reduced. However, the expectation that beneficial insects will not be adversely affected must be verified. The lady beetle is an appropriate indicator organism for non-target acute dietary studies in coleopteran-active Bt corn. However, the parasitic Hymenoptera, *Nasonia vitriprennis*, is not an appropriate indicator species since it is a dipteran parasitoid that does not typically occur in cornfields. The green lacewing study was also inadequate since the Cry34Ab1 and Cry35Ab1 proteins were administered in a moth egg diet that did not allow for exposure to the proteins. Therefore, a carabid (ground beetle) and anthocorid (insidious flower bug) acute dietary toxicity study are needed and will be required by EPA as a condition of registration (personal communication, Mike Mendelsohn).

Dow/Pioneer also collected data from field experiments to assess possible effects of growing line DAS-59122-7 corn on the relative abundance and diversity of certain beneficial insects. Small-scale field studies were conducted in 2001 and 2002 with event PS149B1 expressing the Cry34/35Ab1 binary protein. However, event PS149B1 has lower expression levels of Cry34Ab1 and Cry35Ab1 and since plots were small it is unclear if these field trials present an adequate view about the environmental safety of corn line DAS-59122-7. Additional field trials were conducted in 2003 in York, NE and Johnston, IA with corn line DAS-59122-7. Since larger plots (approximately 4200 ft^2) were used with corn line DAS-59122-7 in the 2003, these data were focused on for an environmental assessment.

Arthropod populations were sampled by visual observations, sticky traps, pitfall traps and litter bags. Predators including chrysopid (lacewings) and coccinellid (lady beetles) egg, larvae and adults were focused on in visual samples. Sticky traps were used to sample for herbivores (aphids, cicadellids (leafhoppers) and thrips), predators (anthocorids (insidious flower bugs), staphylinids (rove beetles), dolichopodids (long-legged flies)) and parasitoids (mymarids (fairyflies)). Pitfall traps and litter bags focused on predators (staphylinids, spiders, centipedes, and carabids (*Pterostichus* spp. and *Harpalus* spp.)) and decomposing organisms (Orabatid mites, Collembola (entomobryids, isotomids, hypogasturids and globular) and millipedes). There were no community-level differences in any of the sample methods between the Bt and non-Bt fields except for a reduction in staphylinds in DAS-59122-7 plots. Since Cry34/35Ab1 is specifically active against chrysomelids and the effect on staphylinids only occurred in the Nebraska site in 2003 and did not occur in the other 2001-2003 field trials, it is assumed that the difference was due to normal field variation. However, Dow/Pioneer intends to continue sampling to verify no effects on staphylinids.

Appendix C of this environmental assessment is a summary table in which corn line DAS-59122-7 is compared to conventional chemical insecticides used to control corn rootworms. The comparison encompasses environmental fate and potential non-target effects. In general, Cry34/Cry35 proteins expressed in corn line DAS-59122-7 compares favorably to these products with respect to the reduced potential for harm in the environment.

Potential impacts on threatened and endangered arthropods:
APHIS coordinates review of petitions with other agencies that have regulatory oversight on that same product. With respect to threatened and endangered species, EPA plays a leadership role in the evaluation. Given the specificity of the Cry34/35Ab1 activity, species outside the insect order Coleoptera should not be affected. EPA has thoroughly examined all threatened and endangered coleopterans that occur in counties where corn is grown, and determined that the breeding habitat of coloepterans does not overlap corn.

Based on a lack of exposure, no unreasonable adverse effects of Cry34/35Ab1 corn to endangered Coleoptera are expected. Many of the endangered and threatened beetles occur in cave or aquatic habitats. None of the endangered beetles are expected to occur in or near cornfields. The American burying beetle (*Nicrophorus americanus*) may occur in old fields or cropland hedge rows. However, based upon the feeding habits of the American burying beetle, it is not expected to occur within corn fields nor will it be exposed to Cry34/35Ab1 proteins. Adult American burying beetles are classified as opportunistic scavengers that feed on anything dead and bury vertebrate carcasses which larvae feed on. Carrion regurgitated by adults is fed to larvae until they are able to feed directly on a carcass.

Environmental fate in soil:
An insect bioassay was conducted in the laboratory with the southern corn rootworm to determine the DT_{50} (time to 50% degradation) of the Cry34/35Ab1 binary protein in soil. A GI_{50} (concentration estimated to reduce growth by 50%) was calculated to determine the DT_{50}. The laboratory bioassay established a DT_{50} of 3.2 days for event PS149B1 (see Petition Section VI.C.2, Table 42). Although the Cry34/35Ab1 expression levels are greater in line DAS-59122-7 than PS149B1, there should not be a significant difference in the DT_{50}. In addition, EPA will require Dow/Pioneer to determine a DT_{50} under field conditions on large-scale plots in different soil-types and environments after commercial release of DAS-59122-7 corn.

E. Potential impacts on biodiversity

Our analysis concludes that line DAS-59122-7 corn exhibits no traits that would cause increased weediness, that its cultivation should not lead to increased weediness of other cultivated corn or other sexually compatible relatives, and it is unlikely to harm non-target organisms common to the agricultural ecosystem or threatened or endangered species recognized by the U.S. Fish and Wildlife Service. Based on this analysis, APHIS concludes that there is no potential for significant impact to biodiversity from a determination of non-regulated status as requested in the petition.

F. Potential impacts on agricultural and cultivation practices

APHIS considered potential impacts associated with the cultivation of rootworm-resistant and glufosinate-ammonium tolerant corn line DAS-59122-7 on current agricultural practices, in particular, those used to control CRW in corn. The potential impact on organic farming was also considered.

Potential impacts of line DAS-59122-7 corn on insect control practices
Dow/Pioneer has provided data which indicate that DAS-59122-7 corn expresses the Cry 34Ab1 and Cry 35Ab1 proteins in root tissues to provide control of corn rootworms. The availability of this product is likely to have an impact on current control practices for corn rootworm that include the use of crop rotation, chemical insecticides, and another Bt corn variety(YieldGard®) that is intended to control corn rootworm. Both crop rotation and the use of chemical insecticides have been important strategies in the past, with commercial use of the YieldGard® variety increasing in the past couple of years since deregulation. However, CRW have developed several

adaptations to control methods including crop rotation and insecticide resistance. Since CRW predominantly oviposit in corn fields, rotating corn with small grains, hay, clover or alfalfa has been utilized as a control method (Levine and Oloumi-Sadeghi 1991). CRW have also been controlled by planting soybean after corn since CRW cannot survive on soybean. Rotating soybeans after corn decreases the need for CRW-targeted insecticide applications. However, wCRW has developed an adaptation to resist the corn/soybean rotation in Illinois and Indiana (Levine and Oloumi-Sadeghi 1996). In areas such as east-central Illinois and northern Indiana, the wCRW has been found to have the ability to lay eggs in soybean, overwinter and hatch the following year in corn (Levine and Oloumi-Sadeghi 1991, Levine and Oloumi-Sadeghi 1996, O'Neal *et al.* 1999, Isard *et al.* 1999, Isard *et al.* 2000).

Northern CRW populations have also developed resistance to the corn/soybean rotation in Minnesota, Iowa, and South Dakota (Gray *et al.* 1998). Prolonged diapause of nCRW involves eggs that remain viable for two winters and hatch two seasons after being laid. Northern CRW have developed the ability for prolonged or extended diapause resulting in a significant proportion of their eggs hatching after two winters leading to an adaptation to rotating corn with crops such as soybean. Extended diapause has been verified in the laboratory from nCRW eggs collected from South Dakota, Minnesota, Illinois and Michigan (Krysan *et al*. 1984, Krysan *et al* 1986, Levine and Oloumi-Sadeghi 1991 Levine *et al*. 1992a, Levine *et al.* 1992b). Field studies conducted by Tollefson (1988) in northwestern Iowa corn fields suggests that extended diapause occurs throughout nCRW distribution in rotated fields. Another study conducted by Levine and Oloumi-Sadeghi (1996) suggests that the wCRW does not demonstrate extended diapause.

In addition to the problem with insect adaptation to crop rotation, many growers simply prefer to grow corn continuously, a practice which necessitates higher inputs of chemical insecticides. As a result of these factors and the very damaging nature of the pest, chemical insecticide usage has increased. The most common chemical regime is the application of a granular insecticide at planting, either banded or in-furrow. In some cases sprays are applied for adult suppression.

Instances of CRW resistance to crop rotation and/or insecticide use typically develop on a local scale which is probably due to limited adult movement before and after mating. In these cases, resistance took at least ten and usually more than 15 years to develop with out implementing IRM strategies. Research is currently underway at the University of Nebraska and USDA-ARS in North Dakota to determine the genetics of esterase-mediated insecticide resistance in wCRW populations. Results of this research are intended to provide knowledge on localized selection and migration that may aid in refining future IRM strategies.

As mentioned earlier, an alternative to crop rotation and insecticide use is the availability of the YieldGard® corn variety which is also an effective tool against corn rootworm. Like chemical control, this corn variety is able to overcome the problems with an extended diapause and lack of crop rotation.

A risk and benefits assessment for reregistration of Bt corn and cotton plant incorporated protectants (PIP's) has been prepared by the EPA (U.S. EPA, 2000) and is posted at the following EPA internet site: http://www.epa.gov/scipoly/sap. Issues considered by the EPA pertaining to this assessment were the subject of a meeting convened on October 18-20, 2000 by the EPA Federal Scientific Advisory Panel (SAP). EPA also convened a SAP meeting, August 27-29, 2002, to consider issues related to corn rootworm-related PIP's. The results of this SAP meeting can be found at: http://www.epa.gov/scipoly/sap/2002/index.htm. Before these new Bt corn varieties were available, farmers were willing to accept lower corn yields, rather than incur the expense, trouble, and uncertain results of chemical insecticide applications to control the target pests. Following the registration of Bt corn varieties in 1995, growers were quick to embrace the new technology. Estimates of Bt corn acreage as a percent of total corn acreage planted increased from 1% in 1996 to 26% in 1999, 2000, and 2002 (USDA NASS, and http://www.usda.gov/nass/pubs/bioc0703.pdf). The USDA National Agricultural extension Service (NASS) statistics compiled from 15 top corn producing states in the Midwest indicate that 30% of this acreage were treated with insecticide registered for corn rootworm control. It is difficult to surmise how much of this application was for the corn rootworm control as these insecticide products used alone or in combination also control other pests such as black cutworms. A 1995 survey conducted in Iowa, the leading corn producing state which accounts for 17.5% of all U.S. production, indicated that growers used chemicals to control CRW 22 % of the time. The most widely used insecticides are from the organophosphate or synthetic pyrethroid classes of chemistry. It is therefore expected that availability of a another practical and economical alternative to chemical insecticides for CRW control would result in a significant reduction in application of such chemicals.

DAS-59122-7 corn could be incorporated into current integrated pest management practices as an additional tool for control. Fields are typically scouted for adult CRW in the late summer or early fall. Economic thresholds are then used in making decisions about control strategies for the following spring planting season. DAS-59122-7 offers an alternative to organophosphate and pyrethroid insecticide applications in cases where thresholds indicate CRW control is needed and the grower chooses to grow corn. No new or specialized equipment or skills would be needed to use the new technology. Reduced pesticide usage by the growers would carry the accompanied benefits of reduced needs for the manufacture, transport, storage and disposal of hazardous chemicals and containers.

In order to delay the potential evolution of resistance in the target pests to Bt Cry proteins expressed in plants, growers have been required by the EPA and/or the developers to implement insect resistance management (IRM) strategies. Dow/Pioneer will submit to EPA, a detailed strategy for approval prior to commercialization of this product. The plan includes monitoring for compliance with the IRM plant, monitoring for resistance to development of resistant CRW populations and mitigation measures if resistant populations are confirmed. Cry3Bb1 corn has been registered by EPA for commercial production since 2002 and there have been no reports of coleopteran insect resistance to the Bt toxin expressed this corn.

Impacts of previously deregulated herbicide tolerant corn on weed control

Several herbicide tolerant corn varieties are commercially available. These were described under Alternative A. The first glufosinate-ammonium tolerant corn varieties were deregulated by APHIS in June 1995. In 1996, prior to the introduction of Roundup Ready (glyphosate herbicide tolerant) corn, pest management data for corn indicate that 1) 3% of acres planted were to herbicide resistant varieties, 2) 83% of pesticide treatments were for weed control, and of those, 20% were post emergence, 39% pre-emergence, and 41% both, 3) mechanical cultivation was used for weed control on 51% of acres planted (Fernandez-Cornejo and Jans 1999). It is estimated that the adoption of other herbicide tolerant corn varieties was associated with an overall decrease in herbicide use in 1996 (especially for the chloroacetamide herbicide family) (Fernandez-Cornejo and McBride, 2000). Nonetheless, in 1997, 96% of the corn acreage in the 10 major corn-producing states was treated with herbicides. At least 18 different herbicide active ingredients have been used, many in combination. Atrazine (which performs well for control of broadleaf weeds) and the chloroacetamides, metolachlor and acetochlor (which perform well for control of annual grass weeds) together accounted for 72% of the total applied in 1997 (Knake 1998; Fernandez-Cornejo and McBride 2000). Overall herbicide use has remained relatively consistent since 1999, with the exception of an increase in glyphosate and mesotrione use and a decrease in metolachlor use (Appendix E). Glyphosate use in corn has increased from 9% in 1999 to 19% in 2003 (NASS, 2000; NASS, 2004). This is consistent with the increased adoption of herbicide-tolerant corn. Herbicide-tolerant corn was planted on 14% of corn acreage in 2003, representing a 75% increase in herbicide-tolerant corn acreage compared to 2001 (Sankula and Blumenthal, 2004). Glufosinate use on corn has remained relatively low since 1999, with use on 2% of corn acreage in 1999 and use on about 3% of corn acreage in 2003 (NASS, 2004; NASS, 2000).

Potential impacts of line DAS-59122-7 corn on weed control

APHIS evaluated data submitted by the petitioners that show that hybrids derived from line DAS-59122-7 corn express PAT that provides the corn with tolerance to glufosinate ammonium herbicides. Line DAS-59122-7 corn, along with glufosinate-ammonium herbicides, is expected to positively impact current agricultural practices used for weed control in a manner similar to other previously deregulated glufosinate-tolerant corn, that is by 1) offering growers a broad spectrum, post-emergent weed control system for both broadleaf and grass weeds; 2) providing the opportunity to continue to move away from pre-emergent herbicides such as metachlor; 3) providing an alternative herbicidal mode of action in corn that allows for improved management of weeds in corn that have developed resistance to herbicides with different modes of action, e.g. triazines and acetolactate synthase (ALS) inhibitors (see *http://www.weedscience.org/Resistance/situation.asp*); and 4) decreasing cultivation needs and increasing the number of no-till acres.

Volunteers of line DAS-59122-7 corn, can be controlled by selective mechanical or manual weed removal or by the use of certain herbicides with active ingredients other than glufosinate ammonium. For example, in soybean, which is the crop most commonly rotated with corn, herbicides based on sulfonylurea, lipid biosynthesis inhibitors, or Fluazifop/fomesafen could be used to control maize volunteers. The commercial introduction and wide adoption in the United States of Roundup Ready soybeans has been associated with an increase in the use of glyphosate to control weeds in soybean, while the use of other herbicides has decreased (Fernandez-Cornejo and McBride, 2000; Heimlich *et al.*, 2000). Glyphosate could also be used to control glufosinate tolerant volunteers of line DAS-59122-7 corn in Roundup Ready soybeans. It is estimated that in 1996, 7% of the total soybean acreage was planted to herbicide tolerant soybeans, compared to an estimated 82% of total soybean acreage planted to herbicide tolerant soybeans in 2003 (Sankula and Blumenthal, 2004). Both glyphosate and glufosinate have relatively low toxicity to humans and wildlife, and do not persist in the environment (Pike, 1999; McGlamery *et al.* 1999).

APHIS considered the possibility that availability and use of glufosinate-tolerant corn lines such as line DAS-59122-7 corn could lead to greater use of glufosinate-ammonium herbicide and result in selection and establishment of weeds tolerant to this herbicide. This would have herbicide use implications both for use of glufosinate tolerant crops previously deregulated by APHIS and possibly for other crops grown in rotation. The occurrence of weeds tolerant to other herbicides is well documented, and technical assistance is available to help identify, prevent, and mitigate this risk (Heap 2000). The risk of glufosinate tolerant weeds developing appears to be quite low. While all herbicides have varying degrees of effectiveness against different weeds, a worldwide survey of herbicide resistant weeds lists seven weed species (as of April 1, 2005) with glyphosate resistance worldwide, of which two are in the United States (http://www.weedscience.org/in.asp). Current practices involving rotation of herbicides with different modes of action and cultivation or mowing to eliminate weeds should be effective in reducing or managing the risk. Because of the lack of cross-resistance between glufosinate-ammonium and glyphosate, the DAS-59122-7 corn could provide an additional alternative for crop rotation. APHIS and the EPA Herbicide Division (Registration Division; Herbicides Branch) have initiated a working group to ensure thorough ongoing considerations of issues surrounding herbicide resistant plants, including the potential for the development of glufosinate tolerant weeds.

Potential impacts on organic farming
It is not likely that organic farmers, or other farmers who choose not to plant transgenic varieties or sell transgenic grain, will be significantly impacted by the expected commercial use of this product since: (a) nontransgenic corn will likely still be sold and will be readily available to those who wish to plant it; (b) farmers purchasing seed will know this product is transgenic because it will be marketed as *Bt Cry34/35Ab1* coleopteran resistant – glufosinate tolerant; and (c) based on the IRM plan, farmers will be educated about recommended management practices. Transgenic corn lines resistant to coleopteran insects, and/or tolerant to glufosinate are already in widespread use by farmers. This particular product should not present new and different issues than those with respect to impacts on organic farmers. APHIS has considered that corn is open-pollinating and it is possible that the engineered genes could move via wind-blown pollen to an adjacent field. All corn, whether genetically engineered or not, can transmit pollen to nearby fields, and a very small influx of pollen originating from a given corn variety does not appreciably change the characteristics of corn in adjacent fields. As described previously in this assessment, the rate of cross-pollination from one field to another is expected to be quite low, even if flowering times coincide. The frequency of such an occurrence decreases with increasing distance from the pollen source such that it sufficiently low at 660 feet away to be considered adequate for production of certified corn seeds. Methods are currently available to prevent or minimize and test for cross-contamination.

G. Potential impacts on raw or processed agricultural commodities.

APHIS analysis of data on agronomic performance, disease and insect susceptibility, and compositional profiles of the kernels indicate no differences between DAS-59122-7 and their non-transgenic hybrid counterparts that would be expected to cause either a direct or indirect plant pest effect on any raw or processed plant commodity from deregulation of line DAS-59122-7.

H. Cumulative Impacts

APHIS considered whether the proposed action could lead to cumulatively significant impacts, when considered in light of other past, present, and reasonably foreseeable future actions, regardless of what agency or person undertakes such actions. In the preceding analysis we have considered the potential for stacking of multiple herbicide tolerance genes, from corn line DAS-59122-7 and other herbicide tolerance genes in previously deregulated transgenic corn lines or in corn developed by other methods, to pose a weed management problem. We have also considered the cumulative impacts of nontransgenic and previously deregulated transgenic herbicide tolerant corn, and other herbicide tolerant crops typically grown in rotation with corn, on the type and toxicity of herbicides and other management practices that can be used to manage weeds in these crops, including the development and management of herbicide tolerant weeds. We have reviewed and considered studies and reports (e.g. U.S. EPA, 2000a; Fernandez-Cornejo and Mcbride, 2000; USDA-ARS, 2003; Sankula and Blumenthal, 2004) to predict the cumulative impacts of deregulation and any subsequent registration and commercialization of another Cry 34/35Ab1 corn, in light of other transgenic coleopteran-resistant Bt plants currently on the market, and the potential for stacking with different lepidopteran resistance genes in hybrids. Considerations included impacts on non-target organisms, changes in pesticides used to control the target pests and other non-targets pests, and the potential for resistance of the Bt toxins to develop as a result of exposure to these toxins in Bt PIPs or in other Bt formulations.

Alternative C, Approval of the Petition in Part

<u>Approval of some but not all of lines requested in the petition</u>. The petition requested a determination of nonregulated status only for lines derived from the one transformation event, designated as DAS-59122-7. Therefore, APHIS can consider only that one line for approval.

<u>Approval of the petition with geographic restrictions</u>. EPA is currently reviewing the application to register DAS-59122-7 corn as a PIP. EPA has completed a thorough analysis of risks to non-target organisms and to threatened and endangered species. After examining all threatened and endangered beetles that occur in counties where corn is grown, they have concluded that none of the beetles= breeding habitats are shared with corn. APHIS has not identified any potential effects from DAS-59122-7 on non-target organisms, including threatened or endangered species, or any adverse impacts on related plant species or plant pest effects that would warrant placing geographic restriction on planting of DAS-59122-7 corn.

VI. <u>CONSIDERATION OF EXECUTIVE ORDERS, STANDARDS AND TREATIES RELATING TO ENVIRONMENTAL IMPACTS</u>

Executive Order (EO) 12898, "Federal Actions to Address Environmental Justice in Minority Populations and Low-Income Populations," requires Federal agencies to conduct their programs, policies, and activities that substantially affect human health or the environment in a manner so as not to exclude persons and populations from participation in or benefiting from such programs. It also enforces existing statutes to prevent minority and low-income communities from being subjected to disproportionately high and adverse human health or environmental effects.

EO 13045, "Protection of Children from Environmental Health Risks and Safety Risks," acknowledges that children may suffer disproportionately from environmental health and safety risks because of their developmental stage, greater metabolic activity levels, and behavior patterns, as compared to adults. The EO (to the extent permitted by law and consistent with the agency's mission) requires each Federal agency to identify, assess, and address environmental health risks and safety risks that may disproportionately affect children.

Each alternative was analyzed with respect to EO 12898 and 13045. None of the alternatives are expected to have a disproportionate adverse effect on minorities, low-income populations, or children. Collectively, the available mammalian toxicity, along with the history of safe use of microbial Bt products and other corn varieties expressing Bt proteins and PAT, establishes the safety of corn line DAS-59122-7 and its products to humans, including minorities, low income populations, and children who might be exposed to them through agricultural production and/or processing. No additional safety precautions would need to be taken. None of the impacts on agricultural practices expected to be associated with deregulation of corn line DAS-59122-7 described above are expected to have a disproportionate adverse effect on minorities, low income populations, or children. As noted above, the cultivation of previously deregulated corn varieties with similar insect resistance and herbicide tolerance traits has been associated with a decrease and/or shift in pesticide applications for those who adopt these varieties that is either favorable or neutral with respect to environmental and human toxicity. If pesticide applications are reduced, there may be a beneficial effect on children and low income populations that might be exposed to the chemicals. These populations might include migrant farm workers and their families, and other rural-dwelling individuals who are exposed to pesticides through ground-water contamination or other means of exposure. It is expected that EPA and USDA Economic Research Service would monitor the use of this product to determine impacts on agricultural practices such as chemical use as they have done previously for Bt products.

EO 13112, "Invasive Species", states that federal agencies take action to prevent the introduction of invasive species and provide for their control and to minimize the economic, ecological, and human health impacts that invasive species cause. Nonengineered corn as well as other Bt and herbicide tolerant corn varieties are widely grown in the United States. Based on historical experience with these varieties and the data submitted by the applicant and reviewed by APHIS, the engineered plant is sufficiently similar in fitness characteristics to other corn varieties currently grown, and it is not expected to have an increased invasive potential.

Executive Order 12114, "Environmental Effects Abroad of Major Federal Actions" requires Federal officials to take into consideration any potential environmental effects outside the U.S., its territories and possessions that result from actions being taken. APHIS has given this due consideration and does not expect a significant environmental impact outside the United States should nonregulated status be determined for corn line DAS-59122-7 or if the other alternatives are chosen. It should be noted that all the considerable, existing national and international regulatory authorities and phytosanitary regimes that currently apply to introductions of new corn cultivars internationally, apply equally to those covered by an APHIS determination of nonregulated status under 7 CFR Part 340. Any international traffic in DAS-59122-7 corn subsequent to a determination of non-regulated status for line DAS-59122-7 would be fully subject to national phytosanitary requirements and be in accordance with phytosanitary standards developed under the International Plant Protection Convention (IPPC).

The purpose of the IPPC "is to secure a common and effective action to prevent the spread and introduction of pests of plants and plant products, and to promote appropriate measures for their control" (http://www.ippc.int/IPP/En/default.htm). The protection it affords extends to natural flora and plant products and includes both direct and indirect damage by pests, including weeds. The IPPC has set a standard for the reciprocal acceptance of phytosanitary certification among the nations that have signed or acceded to the Convention (116 countries as of June, 2001). In April, 2004, a standard for pest risk analysis of living modified organisms (LMOs) was adopted at a meeting of the governing body of the IPPC as a supplement to an existing standard, International Standard for Phytosanitary Measure No. 11 (ISPM-11; Pest Risk Analysis for Quarantine Pests). The standard acknowledges that all LMOs will not present a pest risk, and that a determination needs to be made early in the PRA for importation as to whether the LMO poses a potential pest risk resulting from the genetic modification. APHIS pest risk assessment procedures for bioengineered organisms are consistent with the guidance developed under the IPPC. In addition, issues that may relate to commercialization and transboundary movement of particular agricultural commodities produced through biotechnology are being addressed in other international forums and through national regulations.

The Cartagena Protocol on Biosafety is a treaty under the United Nations Convention on Biological Diversity (CBD) that established a framework for the safe transboundary movement, with respect to the environment and biodiversity, of LMOs, which includes those modified through biotechnology. The Protocol came into force on September 11, 2003 and 119 countries are parties to it as of April 14, 2005 (see http://www.biodiv.org/biosafety/default.aspx). Although the United States is not a party to the CBD, and thus not a party to the Cartagena Protocol on Biosafety, US exporters will still need to comply with domestic regulations that importing countries that are parties to the Protocol have put in place to comply with their obligations. The first intentional transboundary movement of LMOs intended for environmental release (field trials or commercial planting) will require consent from the importing country under an advanced informed agreement (AIA) provision, which includes a requirement for a risk assessment consistent with Annex III of the Protocol, and the required documentation. LMOs imported for food, feed or processing (FFP) are exempt from the AIA procedure, and are covered under Article 11 and Annex II of the Protocol. Under Article 11 Parties must post decisions to the Biosafety Clearinghouse database on domestic use of LMOs for FFP that may be subject to transboundary movement. To facilitate compliance with obligations to this protocol, the US Government has developed a website that provides the status of all regulatory reviews completed for different uses of bioengineered products (http://usbiotechreg.nbii.gov). This data will be available to the Biosafety Clearinghouse.

APHIS continues to work toward harmonization of biosafety and biotechnology consensus documents, guidelines and regulations, including within the North American Plant Protection Organization (NAPPO), which includes Mexico, Canada, and the United States and in the Organization for Economic Cooperation and Development. NAPPO has completed three modules of a standard for the *Importation and Release into the Environment of Transgenic Plants in NAPPO Member Countries* (see http://www.nappo.org/Standards/Std-e.html). APHIS also participates in the North American Biotechnology Initiative (NABI), a forum for information exchange and cooperation on agricultural biotechnology issues for the U.S., Mexico and Canada. In addition, bilateral discussions on biotechnology regulatory issues are held regularly with other countries including: Argentina, Brazil, Japan, China, and Korea. Many countries, e.g. Argentina, Australia, Canada, China, Japan, Korea, Philippines, South Africa, Switzerland, the United Kingdom, and the European Union have already approved Bt corn varieties to be grown or imported for food or feed (http://www.agbios.com/dbase.php).

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Appendix A. USDA Acknowledged and Approved Field Tests with Bt *cry34/35Ab1* Corn Line DAS-59122-7 listed by Permit (r) or Notification (n) Number.

i. Dow AgroSciences
  a. 01-277-06n
  b. 02-060-21n
  c. 02-060-22n
  d. 02-060-23n
  e. 02-162-08n
  f. 03-008-06n
  g. 03-035-15n
  h. 03-035-16n
  i. 03-052-09n

ii. Pioneer HiBred
  a. 01-022-04r
  b. 02-023-02r
  c. 03-022-01r
  d. 03-022-02r

Appendix B. Potential for introgression from *Zea mays* to its sexually compatible relatives.

Wild diploid and tetraploid members of *Zea* collectively referred to as teosinte are normally confined to the tropical and subtropical regions of Mexico, Guatemala, and Nicaragua. A few isolated populations of annual and perennial teosinte have been reported to exist in Florida and Texas, respectively (USDA-APHIS, 1998b); but local botanists and agronomists familiar with the flora of these regions have not documented any current populations of teosinte there (U.S. EPA, 2000a, see page IIC5). The Mexican and Central America teosinte populations primarily exist within and around cultivated maize fields; they are partially dependent on agricultural niches or open habitats, and in some cases are grazed upon or fed to cattle which distribute the seed. While some teosinte may be considered to be weeds in certain instances, they are also used by some farmers for breeding improved maize (Sánchez and Ruiz, 1997, and references therein).

All teosinte members can be crossed with cultivated corn to produce fertile F_1 hybrids (Doebley, 1990a; Wilkes, 1967; and Jesus Sánchez, personal communication, 1998). In areas of Mexico and Guatemala where teosinte and corn coexist, they have been reported to produce hybrids. Of the annual teosinte, *Z. mays* ssp *mexicana* forms frequent hybrids with maize, *Z. luxurians* hybridizes only rarely with maize, whereas populations of *Z. mays* ssp. *parviglumis* are variable in this regard (Wilkes, 1977; Doebley, 1990a). Fewer fertile hybrids are found between maize and the perennial *Z. perennis* than are found with Z. *diploperennis* (J. Sánchez, personal communication, 1998). Research on sympatric populations of maize and teosinte suggests introgression has occurred in the past, in particular from maize to *Z. mays* ssp. *luxurians* and *Z. mays* ssp. *diploperennis* and from annual Mexican plateau teosinte (*Z. mays* ssp. *mexicana*) to maize (Kato, 1997 and references therein). Nonetheless, in the wild, introgressive hybridization from maize to teosinte is currently limited, in part, by several factors including distribution, differing degrees of genetic incompatibility, differences in flowering time in some cases, block inheritance, developmental morphology and timing of the reproductive structures, dissemination, and dormancy (Doebley, 1990a; Galinat, 1988). First-generation hybrids are generally less fit for survival and dissemination in the wild, and show substantially reduced reproductive capacity which acts as a significant constraint on introgression. Teosinte has coexisted and co-evolved in close proximity to maize in the Americas over thousands of years, but maize and teosinte maintain distinct genetic constitutions despite sporadic introgression (Doebley, 1990a).

The genus *Tripsacum* contains up to 16 recognized species, most of which are native to Mexico, Central and South America. But three *Tripsacum* species, *T. floridanum*, *T. lanceolatium*, and *T. dactyloides*, exist as wild and/or cultivated in the U.S. (Hitchcock, 1971). Though many of these species occur where corn might be cultivated, gene introgression from line 1507 corn under natural conditions is highly unlikely or impossible. Hybrids of *Tripsacum* species with *Zea* are difficult to obtain outside of a laboratory and are often sterile or have greatly reduced fertility, and none are able to withstand even the mildest winters (Beadle, 1980; Galinat, 1988).

References (see EA, Literature Cited, Section VII.)

Appendix C. Environmental and human health safety of Cry 34Ab1 and Cry 35 Ab1 (as expressed in corn line DAS-59122-7 or as purified from a microbial source) compared to other common insecticides used on corn to control the corn rootworm target pests, and other non-target pests.

	Cry34/35Ab1	Cry3Bb1	Terbufos (Counter®)	Tefluthrin (Force®)
Environmental Fate	The Dt_{50} estimate for Cry34/35Ab1 binary protein in soil was found to be 3.2 days. (1)	The maximum environmental concentration for organisms feeding on corn plants is predicted to be 93 μg/g based on the highest Cry3Bbl.11098 expression level measured in pollen and leaf tissue of MON 863 corn plants. The maximum environmental concentration for soil dwelling organisms is predicted to be 13.3 mg/kg based on the assumption that corn plants are tilled into the top 6" of soil at the time of maximum leaf expression for Cry3Bbl .11098 (*i.e.* 93 ptg/g). (2) The DT_{50} and DT_{90} estimates for Cry3Bb1 protein in soil were found to range from 0.9 to 2.3 days and 7.4 to 50 days respectively. (2)	Terbufos hydrolyzes at pH 5, 7, and 9 with a half-life of 2.2 weeks. Formaldehyde was the major degradate detected in this study. Aerobic soil metabolism study indicate that terbufos degrades in silt loam soil with a half-life of 26.7 days. The major degradates detected in this study included carbon dioxide, terbufos sulfoxide, and terbufos sulfone. Terbufos residues have a half-life of less than 40 days in field plots of loam soil treated with a 15 percent granular formulation at an application rate of 1 lb ai/A. The sampling protocol was inadequate to accurately assess the dissipation of terbufos residues in field soil and a new study is required. The available data reviewed by the Agency are not sufficient to fulfill data requirements nor to assess the environmental fate of terbufos. EPA is concerned about the potential for the two degradates, terbufos sulfoxide and sulfone, to leach to groundwater, and the potential for parent terbufos and the sulfoxide and sulfone degradates to runoff to surface water. Terbufos parent degrades rapidly to the sulfoxide and sulfone metabolites, and is considered moderately mobile.	Tefluthrin is immobile in soil and, therefore, will not leach into ground water. Additionally, due to the insolubility and lipophilic nature of tefluthrin, any residues in surface water will rapidly and tightly bind to soil particles and remain with sediment, therefore not contributing to potential Tefluthrin is immobile in soil and, therefore, will not leach into ground water. Additionally, due to the insolubility and lipophilic nature of tefluthrin, any residues in surface water will rapidly and tightly bind to soil particles and remain with sediment, therefore not contributing to potential dietary exposure from drinking water. Plant metabolism studies indicate that tefluthrin per se is not translocated to plants but is degraded in soil to two principal metabolites that are capable of being taken up by plants. EPA has decided that Metabolite VI need not be regulated. Based on tefluthrin not being registered for residentia1 non-food sites, EPA concludes that the aggregate short- and intermediate-term risks do not exceed levels of concern (MOE 1ess than 100), and that there is reasonable certainty that no harm will result from aggregate exposure to

			Terbufos sulfoxide and sulfone are more mobile and persistent than parent terbufos. The acute DWLOCs calculated for the general U.S. population is 8.1 Fg/L. The chronic DWLOCs calculated for the general U.S. population is 1.7 Fg/L. Maximum acute and chronic estimated environmental concentrations (EECs) for parent terbufos plus the sulfoxide and sulfone degradates exceed the acute and chronic DWLOCs, respectively, in all cases. (5)	tefluthrin residues. (8)
Avian toxicity	Feeding Cry34/35Ab1 grain from event 15344 to chicken broilers resulted in no adverse effects on mortality, weight gain, feed efficiency and carcass yields. (1) LC_{50} ICP >25.1 ng active ingredient/mg diet LC_{50} Cry34Ab1 >23 ng active ingredient/mg diet LC_{50} Cry35Ab1 >2.1 ng active ingredient/mg diet (1)	Feeding of Cry3Bb-containing grain to Bobwhite quail at 10 and 35% of their diets, respectively, resulted in no adverse effects on growth or survival (2). The dietary LC_{50} value for Cry3Bb1 corn grain to juvenile Northern Bobwhite was greater than 70,000 ppm (10% of the diet) in eight day observation. (3)	Seven incidents to nontarget terrestrial organisms have been reported. Up to three of the incidents had some indication of misuse or misapplication. All the mortalities involved bird species (mostly raptors), with the exception of one incident involving red wolves in North Carolina, which is believed to be the result of an intentional poisoning. Calculated RQs for birds and mammals significantly exceed EPA's risk concern for both granular formulations. (5) Dietary Avian Toxicity: 143 and 157 ppm (from two bobwhite studies). - Avian Reproduction: Terbufos was not considered to produce avian reproductive effects based on results of a bobwhite quail study and a mallard duck study. (6)	Low toxicity to birds (9).
Fish toxicity	Feeding Cry34Ab1 microbially-produced protein and Cry35Ab1 microbially produced	Feeding of Cry3Bb-containing grain to channel catfish at 10 and 35% of their diets,	EPA has concerns about risk to nontarget aquatic organisms from parent terbufos and the terbufos	Highly toxic to fish (9)

	insect control protein to rainbow trout resulted in no adverse effects. (1) LC_{50} ICP >100 mg active ingredient/kg diet LC_{50} Cry34Ab1 >25 mg active ingredient/kg diet LC_{50} Cry35Ab1 >75 mg active ingredient/kg diet (1)	respectively, resulted in no adverse effects on growth or survival. (2)	sulfoxide and sulfone degradates based on widespread fish kill incidents involving terbufos use on corn with all application methods. These concerns are further supported by standard LOC criteria, which indicate risk concerns to aquatic fish and invertebrates associated with both the clay-based (15% active ingredient) and polymer-based (20% active ingredient) granular formulations using banded applications.(5) Terbufos ranks fourth in pesticide-induced fish kills reported to the Agency, and is the leading cause of fish kills from use on corn. **Freshwater Fish Acute Toxicity**: Ranges from 0.77 to 20.00 ppb. - Freshwater Invertebrate Acute Toxicity: 0.31 ppb for Daphnia magna. - Marine/Estuarine Fish Acute Toxicity: Data gap.- Marine/Estuarine Invertebrate Toxicity: Data gap. Mollusk toxicity: Data gap (5)	
Nontarget and beneficial organisms	Cry34Ab1 and Cry35Ab1 microbially produced protein were fed to non-target insects and resulted in no adverse effects. (1) **Green lacewing:** LC_{50} ICP >280 µg active ingredient/mL diet; LC_{50} Cry34Ab1 >160 µg active ingredient/mL diet; LC_{50} Cry35Ab1 >120 µg active ingredient/mL diet (1) **Parasitic Hymenoptera:** LC_{50} ICP >280 µg active ingredient/mL diet; LC_{50} Cry34Ab1 >160 µg active	Various "nontarget" organisms were exposed to high doses of leaf tissue, grain or pollen expressing Cry3Bb or to an artificial diet containing the purified protein for varying periods of time. They demonstrate that Cry3Bbl.11098 protein in MON 863 poses no significant risk for harm to nontarget organism populations. (2) **Green lacewing:** LC_{50} >8,000 ppm Cry3Bb1	Terrestrial Field Study (Level 1): both soil-incorporated (2 lb ai/A) and nonsoil-incorporated (1 lb/A) resulted in nontarget mortalities, with the latter application much more severe in its effects (5,6)	Data not found.

	ingredient/mL diet; LC_{50} Cry35Ab1 >120 µg active ingredient/mL diet (1) **Lady beetle:** LC_{50} ICP >280 µg active ingredient/mL diet; LC_{50} Cry34Ab1 >160 µg active ingredient/mL diet; LC_{50} Cry35Ab1 >120 µg active ingredient/mL diet (1) ***Daphnia magna***: EC_{50} ICP >100 mg active ingredient/mL diet; LC_{50} Cry34Ab1 >57 mg active ingredient/mL diet; LC_{50} Cry35Ab1 >43 mg active ingredient/mL diet (1)	protein (3) **Parasitic Hymenoptera:** LC_{50} >400 ppm Cry3Bb1 protein (3) **Lady beetle:** LC_{50} for adult *H. convergens* is >8,000 µg Bt protein/mL diet**;** LC_{50} for *C. maculate* >93 µg/g fresh pollen weight (3) ***Daphnia magna*:** LC_{50} >120 mg pollen/L (3)		
Honeybee toxicity	Honey bees were fed Cry34/35Ab1 pollen from event TC5639 and Cry34Ab1 and Cry35Ab1 microbially produced protein. (1) LC_{50} pollen >2 mg/larvae (0.056 µg Cry34/35Ab1 ICP/larvae) LC_{50} ICP >20 µg active ingredient/larvae LC_{50} Cry34Ab1 >3.2 µg active ingredient/larvae LC_{50} Cry35Ab1 >2.4 µg active ingredient/larvae (1)	**Adult Honey Bee:** Cry3Bbl.11231 in an artificial diet LC_{50} >360 µg/ml in diet NOEC > 3X predicted maximum Cry3Bbl concentration in pollen **Larval Honey Bee :** Cry3Bbl.11231 in water NOEC 2 1790 ug/ml in diet LC_{50} > 10X predicted maximum Cry3Bb I concentration in pollen. (2, 3)	Not described in available studies.	High toxicity to bees (10)
Mammalian toxicity	Cry34Ab1 and Cry35Ab1 microbially produced protein were fed to mice and no acute oral toxicity or adverse effects in terms of body weight, detailed clinical observations and gross-pathological lesions were observed. (1) LD_{50} >2700 mg Cry34Ab1/kg	**Acute toxicity**: Bt is practically non-toxic to humans and animals. Humans exposed orally to 1000 mg/day of Bt showed no effects.. No oral toxicity was found in rats, or mice fed protein crystals from Bt var. israelensis. The LD50 is greater than 5000 mg/kg for the Bt product Javelin	**Acute Oral**: Toxicity Category I (1.6 and 1.3 mg/kg for male and female rats, respectively). - **Acute Dermal**: Toxicity Category I (0.81 and 0.93 mg/kg for male and female rabbits, respectively). - **Acute Inhalation**: Toxicity Category I (< 0.2 mg/L).	**Acute toxicity** studies with the technical grade of the active ingredient tefluthrin: oral LD50 in the rat is 21.8 mg/kg for males and 34.6 mg/kg for females; dermal LD50 in the rat is 316 mg/kg in males and 177 mg/kg in females; acute inhalation LC50 in the rat is 0.037 mg/l and 0.049 mg/l in

	LD_{50} >1850 mg Cry35Ab1/kg LD_{50} >2000 mg Cry34/35Ab1/kg (1)	in rats and greater than 13,000 mg/kg in rats exposed to the product Thuricide. Single oral dosages of up to 10,000 mg/kg did not produce toxicity in mice, rats, or dogs. The dermal LD50 for a formulated Bt product in rabbits is 6280 mg/kg. A single dermal application of 7200 mg/kg of Bt was not toxic to rabbits. Bt is an eye irritant; 100 grams of formulated product applied in congestion of the iris as well as redness and swelling. **Chronic toxicity**: No complaints were made by 8 men after they were exposed for 7 months. **Dietary administration** of Bt for 13 weeks to rats at dosages of 8400 mg/kg/day did not produce toxic effects. Some reversible abnormal redness of the skin was observed when 1 mg/kg/day of formulated Bt product was put on scratched skin for 21 days. No general, systemic poisoning was observed **Reproductive effects**: No indication. **Teratogenic effects**: No evidence. **Mutagenic effects**: There is no evidence of mutagenicity in mammalian species. **Carcinogenic effects**: It is unlikely that Bt is carcinogenic. (4).	- **Delayed Neurotoxicity**: No evidence of acute delayed neurotoxicity at the 40 mg/kg dosage level tested in hens. - **Subchronic Feeding**: The NOEL for both systemic effects and cholinesterase inhibition in a rat subchronic study is 0.25 ppm. - **Subchronic Dermal**: The NOEL for systemic effects in a 30-day rabbit study is 0.020 mg/kg. - **Mutagenicity**: Terbufos did not exhibit mutagenic potential in the Ames assay, the in vivo cytogenetic assay, and the dominant lethal test. - **Teratogenicity**: The NOEL for developmental toxicity in a rat teratology study is 0.1 mg/kg/day. - **Reproduction**: The NOEL for reproductive effects in a three-generation rat reproduction study is 0.25 ppm. - **Oncogenicity**: None (5,6)	male and female rats, respectively; primary dermal irritation study in the rabbit showed slight irritation; and the acute delayed neurotoxicity study did not show acute delayed neurotoxicity. In an oral toxicity study, the NOEL for female rats is 100 ppm (equivalent to approximately 5 mg/kg/day). The NOEL for skin effects in rats is 1.0 mg/kg). The NOEL for neurological effects (the observed postural effects) may be between 0.025 and 0.1 mg/kg. **Carcinogenicity**: There was no evidence of carcinogenic potential. **Mutagenicity**: There is no mutagenicity concern. **Metabolism**: In both rats and dogs, when given either 1 or 10 mg/kg, most of the radioactivity was found in the feces unchanged and most urinary metabolites were conjugated. In rats, the halflife in the liver is 4.8 days, in the fat is 13.3 days and in the blood is 10.6 days. In a study with rat fat, half of the radioactive residues could be attributed to the parent and the remaining residues consisted of a mixture of fatty acid esters of hydroxylated parent metabolites. **Neurotoxicity**: No acceptable mammalian neurotoxicity studies (8). are available.(8)
Nontarget soil organism effects	Cry34Ab1 and Cry35Ab1 microbially produced protein were fed to Collembola and earthworms and no adverse effects were	The maximum environmental concentration for soil dwelling organisms is predicted to be 13.3 mg/kg based on the	Not described by present reports.	Not found in these reports

	observed. (1) **Collembola:** LC_{50} ICP >12.7 mg active ingredient/kg diet; LC_{50} Cry34Ab1 >3.2 mg active ingredient/kg diet; LC_{50} Cry35Ab1 >9.5 mg active ingredient/kg diet (1) **Earthworms:** LC_{50} ICP >25.4 mg active ingredient/kg diet; LC_{50} Cry34Ab1 >6.4 mg active ingredient/kg diet; LC_{50} Cry35Ab1 >19.0 mg active ingredient/kg diet (1)	assumption that corn plants are tilled into the top 6" of soil at the time of maximum leaf expression for Cry3Bbl .11098 (*i.e.* 93 ptg/g). The measured NOECs from these tests exceed the maximum predicted environmental concentration by 3- to 140-fold, demonstrating an adequate margin of safety for these organisms (2). **Collembola:** LC_{50} >872.5µg protein (50% corn leaf tissue in the diet)m (3) **Earthworms:** LC_{50} 570 mg Cry3Bb1 protein/kg dry soil (3)		
Toxicity	Not assigned	Class III	Classified by EPA as Toxicity Category I	Toxicity class I for dermal, oral, inhalation exposures, and Class IV for skin irritation.
EDF=s Integrated Environmental Rankings - Combined human & ecological scores (7)	Not ranked	Not ranked	85-100% where 0 is the lowest and 100 is the highest hazard rating (7).	Data lacking; not ranked by any system in Scorecard.

Source of information:

1. Petition for Determination of Nonregulated Status for *Bt* Cry34/35Ab1 Insect-Resistant, Glufosinate-Tolerant Corn: Corn Line 59122-7.
2. Petition for Determination of Nonregulated Status for the Regulated Article: Corn Rootworm Protected Corn Event MON 853 (2000), Monsanto Company, St. Louis, MO.
3. EPA Biopesticide Registration Action Document (BRAD) http://www.epa.gov/pesticides/biopesticides/pips/bt_brad.htm
4. Extoxnet: Extension Toxicology Network, Pesticide Information Profiles http://ace.ace.orst.edu/info/extoxnet/pips/bacillus.htm
5. Overview of Revised Terbufos Risk Assessment, Office of Pesticide Programs-- US EPA http://www.epa.gov/pesticides/op/terbufos/terbufosview.htm
6. EPA Pesticide Fact Sheet http://pmep.cce.cornell.edu/profiles/insect-mite/propetamphos-zetacyperm/terbufos/insect-prof-terbufos.html
7. Environmental Defense Fund Scorecard. http://www.scorecard.org/chemical-profiles/
8. Tefluthrin; Pesticide Tolerance ENVIRONMENTAL PROTECTION AGENCY (40 CFR Part 180) [Federal Register: November 26, 1997 (Volume 62, Number 228)

http://www.epa.gov/fedrgstr/EPA-PEST/1997/November/Day-26/p30946.htm
9. Farm Chemicals Handbook, p. C374.
10. Ohio State University, Insect Pests of Field Crops Bulletin 545 AToxicity of Pesticides@ http://www.ag.ohio-state.edu/b45/b45_48.html

Appendix D. Data submitted with the petition in support of nonregulated status for *Bt cry 34Ab1 and cry 35Ab1* corn line DAS-59122-7

Molecular Genetic Characterization
Southern analysis of the *cry34Ab1* gene in DAS-59122-7, Fig. 4, pg. 34
Southern analysis of the *cry35Ab1* gene in DAS-59122-7, Fig. 5, pg. 35
Southern analysis of the *pat* gene in DAS-59122-7, Fig. 6, pg. 36
Southern analysis of the *cry34Ab1* gene in DAS-59122-7, Fig. 7, pg. 37
Southern analysis of the *cry34Ab1* gene in DAS-59122-7, Fig. 8, pg. 40
Southern analysis of the *cry35Ab1* gene in DAS-59122-7, Fig. 9, pg. 41
Southern analysis of the *pat* gene in DAS-59122-7, Fig. 10, pg. 42
Southern analysis of the *cry35Ab1* gene in DAS-59122-7, Fig. 11, pg. 43
Southern analysis of the *pat* gene in DAS-59122-7, Fig. 12, pg. 44
Southern analysis of the *cry34Ab1* gene in DAS-59122-7, Fig. 13, pg. 51
Southern analysis of the *cry35Ab1* gene in DAS-59122-7, Fig. 14, pg. 52
Southern analysis of the *pat* gene in DAS-59122-7, Fig. 15, pg. 53
Southern analysis of the ubiquitin promoter gene in DAS-59122-7, Fig. 16, pg. 54
Southern analysis of the perox promoter in DAS-59122-7, Fig. 17, pg. 55
Southern analysis of the 35S promoter in DAS-59122-7, Fig. 18, pg. 56
Southern analysis of the ubiquitin intron DAS-59122-7, Fig. 19, pg. 57
Southern analysis of the pin II terminator in DAS-59122-7, Fig. 20, pg. 58
Southern analysis of the *cry34Ab1* in DAS-59122-7, Fig. 21, pg. 59

Southern analysis of the *pat* gene in DAS-59122-7, Fig. 23, pg. 61
Southern analysis of the ubiquitin promoter in DAS-59122-7, Fig. 24, pg. 62
Southern analysis of the TA perox promoter in DAS-59122-7, Fig. 25, pg. 63
Southern analysis of the 35S promoter in DAS-59122-7, Fig. 26, pg. 64
Southern analysis of the ubiquitin promoter in DAS-59122-7, Fig. 27, pg. 65
Southern analysis of the pin II terminator in DAS-59122-7, Fig. 28, pg. 66
Southern analysis of the *spc* probe for DAS-59122-7 corn, Fig. 29, pg. 68
Southern analysis of the *spc* probe in DAS-59122-7 corn, Fig. 30, pg. 69
Southern analysis of the *tet* probe DAS-59122-7 corn, Fig. 31, pg. 70
Southern analysis of the *tet* probe in DAS-59122-7 corn, Fig. 32, pg. 71
Southern analysis of the *VirG* gene DAS-59122-7 corn, Fig. 33, pg. 72
Southern analysis of the LB backbone probe in DAS-59122-7 corn, Fig. 34, pg. 73
Southern analysis of the LB backbone probe in DAS-59122-7 corn, Fig. 35, pg. 74
Southern analysis of the RB backbone probe in DAS-59122-7 corn, Fig. 36, pg. 75
Mendelian segregation of Bt Cry34/35Ab1 corn line DAS-59122-7, Table 13, pg. 98
Cry34Ab1 protein levels in tissues from line 59122-7 hybrids by ELISA, Table 21, pg. 125
Cry35Ab1 protein levels in tissues from line 59122-7 hybrids by ELISA, Table 22, pg. 126
PAT protein levels in tissues form line 59122-7 hybrids by ELISA, Table 24, pg. 128
Phenotypic Characterization and Evidence to Support a Lack of Unintended Effects
Efficacy Data: resistance to Coleopteran pests (Petition, Section V.F.1)
Agronomic Performance Traits: between a line 59122-7 hybrid, a near isogenic Cry1F hybrid, a non-transgenic near isogenic line (BC2S1) and an elite non-transgenic near isogenic line in various field trials conducted in the U.S. (Petition, Section V.F.2)
Compositional and Nutritional Analysis: whole-plant forage data on proximate analysis (for protein, fat, fiber, ash and carbohydrates). Grain data on proximate analysis, mineral analysis, fatty acid composition,

amino acid analysis, vitamin content and antinutrient content (phytic acid and trypsin inhibitor). (Petition, Section, V.E).
Analysis of Nontarget Effects
Comparison of maize-derived Cry 34/Ab1 proteins and microbially-derived Cry 34/35 Ab1 proteins used for bioassays, N-terminal sequence analysis, glycosylation and biological activity, see petition section V.D.1, pgs 102-116.
Environmental Fate of Cry 34/35Ab1 proteins in Soil, see petition section VI.C.2, pgs. 164-165.
Colembola – 28 day chronic exposure study: CBI, Appendix 34. Also see petition page 159
Honeybee – Evaluation of dietary exposure on honeybee development: CBI Appendix 25. Also see petition page160.
Green Lacewing Larvae – Dietary toxicity: Appendix 35. Also see petition page 160.
Parasitic Hymenoptera – Dietary toxicity: CBI, Appendix 33. Also see petition page 160.
Ladybird Beetle – Dietary toxicity: CBI, Appendix 10. Also see petition page 160.
Daphnia magna – Acute toxicity: CBI, Appendix 32. Also see petition page 159
Earthworm – Acute toxicity; CBI, Appendix 27. Also see petition page 159.
Broiler Chicken – Nutritional Equivalency: CBI, Appendix 9. Also see petition page 158.
Rainbow Trout – 8-day dietary exposure: Appendix 31. Also see petition page 159.
Resistance Management Plan – See page 178 of petition.
Mice – Acute oral toxicity: CBI, Appendix 23. See also petition page 156.
Allergenicity Profile – comparison of amino acid sequence similarity of Cry34Ab1, Cry35Ab1 and PAT proteins to known allergens: CBI, Appendix 24. See also petition page 153.
In vitro digestibility of PSB149B1 proteins – CBI, Appendix 19. See also petition page 153.

Appendix E. Changes in Herbicide Use in Corn Since 1999-2003

Herbicide	**1999[b]**		**2003[a]**		**% Change[d]**
	% Area Treated	**Total Applied[c]**	**% Area Treated**	**Total Applied[c]**	
Acetochlor	27	31,824	26	36,067	13.3
Atrazine	70	54,780	68	55,642	1.5
Glufosinate-Ammonium	*	*	3	833	-
Glyphosate	9	4,162	19	11,913	186.2
Mesotrione	*	*	13	976	-
Metolachlor	29	29,554	6	6,384	-78.4
Nicosulfuron	15	150	11	166	10.6
States Surveyed	CO, IL, IN, IA, KS, KY, MI, MN, MO, NE, NC, OH, SD, TX, WI		CO, IL, IN, IA, KS, KY, MI, MN, MO, NE, NY, NC, ND, OH, PA, SD, TX, WI		

[a] USDA-NASS. 2004. Agricultural Chemical Usage 2003 Field Crops Summary.
[b] USDA-NASS. 2000. Agricultural Chemical Usage 1999 Field Crops Summary
[c] 1000 lbs/acre
[d] Percent change in total lbs applied
[*] No data available for that growing season