Approval of Mycogen Seeds c/o Dow AgroSciences LLC Request (03-181-01p) Seeking Extension of Determination of Non-regulated Status For Bt Cry1F Insect Resistant, Glufosinate Tolerant Corn Line 6275

Finding of No Significant Impact

The Animal and Plant Health Inspection Service (APHIS), United States Department of Agriculture (USDA), has prepared an environmental assessment (EA) prior to approving an extension (APHIS Number 03-181-01p) of the determination of nonregulated status granted for petition 00-136-01p received from Mycogen Seeds c/o Dow AgroSciences LLC under APHIS regulations at 7 CFR Part 340. The subjects of extension request 03-181-01p are genetically engineered to express two foreign proteins, a truncated Cry1F insecticidal protein and a phosphinothricin-N-acetyltransferase enzyme, which confer resistance to certain lepidopteran insect pests and tolerance to glufosinate herbicide, respectively. Based on the analysis carried out in the EA, APHIS has reached a finding of no significant impact (FONSI) to the environment from its determination that event TC6275 shall no longer be considered a regulated article. Before reaching this decision, APHIS requested and considered comments on the EA from the public. A response to the one comment received is included as an attachment to this FONSI statement.

Cindy Smith
Deputy Administrator
Biotechnology Regulatory Services
Animal and Plant Health Inspection Service
U.S. Department of Agriculture
Date: 10-20-04

Attachment
Finding of No significant Impact
response to Comments
APHIS No. 03-181-01p

In response the notice published in the *Federal Register* on August 17, 2004 (69 FR 51058-51059), APHIS received one comment on the environmental assessment (EA) prepared for APHIS No. 03-181-01p, a request for extension of a determination of nonregulated status from Mycogen Seeds c/o Dow AgroSciences LLC for corn line 6275. The comment, from a private individual, opposed the extension request based on allegations that *Bacillus thuringiensis* is hazardous to humans and that line 6275 contained plant pathogens. The comment of the individual does not provide data to support the allegations. We have confined our response to points made by the commenter that relate to any plant pest or environmental risks posed by the subject extension of a determination of nonregulated status.

APHIS addresses, in the EA, the toxicity of Cry1F in humans as well as other non-target organisms. The Cry1F protein expressed in line 6275 corn is similar to the well known Cry1A class of lepidopteran-specific toxins produced by Bt strains. The specificity of the insecticidal activity of these Cry proteins appears to be dependent upon their binding to specific receptors present in the mid-gut of lepidopteran insects (Lambert, *et al.*, 1996; Van Rie *et al.*, 1990; Van Rie *et al.*, 1989; Hofmann *et al.*, 1988a and 1988b; and Wolfersberger *et al.*, 1986). These insecticidal proteins are not expected to adversely effect other invertebrates and all vertebrate organisms, including non-target birds, mammals and humans, because these organisms would not be expected to contain the receptor protein found in the insect's midgut. APHIS evaluated laboratory and field studies on representative species that support these expectations.

APHIS also addresses, in the EA, the integration of plant pathogen DNA in corn line 6275 and concluded that corn line 6275 exhibits no plant pathogenic properties. Although DNA from pathogens were used in its development, these plants are not infected by these organisms, nor can these plants incite disease in other plants.

USDA/APHIS Decision on Mycogen Seeds c/o Dow AgroSciences LLC Request 03-181-01P Seeking an Extension of a Determination of Non-regulated Status for Bt Cry1F Insect Resistant, Glufosinate Tolerant Corn Line 6275

Environmental Assessment

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I. SUMMARY

The Animal and Plant Health Inspection Service (APHIS), U.S. Department of Agriculture (USDA), has prepared an environmental assessment (EA) in response to a request (APHIS No. 03-181-01p) from Mycogen Seeds c/o Dow AgroSciences LLC (hereafter referred to as Mycogen/Dow), Indianapolis, IN, for an extension of a previous determination of nonregulated status issued for insect-resistant, glufosinate-tolerant corn line 1507, the antecedent organism in APHIS No. 00-136-01p. The Mycogen/Dow extension request states that insect-resistant, glufosinate-tolerant corn line 6275 (OECD designation number DAS-06275-8) is similar to the antecedent organism and therefore does not present a plant pest risk and should no longer be a regulated article under the regulations in 7 CFR part 340. Corn line 6275 is currently considered a regulated article under the regulations at 7 CFR part 340; therefore importation, interstate movement, and field tests of this corn must be conducted under authorizations from APHIS.

Corn lines 6275 and 1507 both have been engineered to express foreign versions of the Cry1F insecticidal protein and the phosphinothricin-acetyltransferase enzyme, which confers the herbicide tolerance trait, but the genetic constructs are slightly different. The 6275 corn has been genetically modified to express a modified maize-optimized version of the *cry1F* gene from *Bacillus thuringiensis (Bt)* subsp. *aizawa*i that protects the corn plants against the feeding damage of certain lepidopteran insect larvae including European corn borer (ECB; *Ostrinia nubilalis*), southwestern corn borer (SWCB; *Diatraea grandiosella*), fall armyworm (FAW; *Spodoptera frugiperda*) and black cutworm (*Agrostis ipsilon*). As with the previous Bt Cry proteins, Cry1F has a high degree of specificity for the target pests. Line 6275 corn also expresses the *bar* gene which is derived from the bacterium *Streptomyces hygroscopicus*, whereas corn line 1507 expresses the *pat* gene, which is derived from Streptomyces *viridochromogenes.* Both *pat* and *bar* genes encode an enzyme phosphinothricin-N-acetyltransferase that detoxifies glufosinate and thereby confers tolerance to herbicides based on this active ingredient. The herbicide tolerance provides an alternative weed management tool for farmers and a method of selecting for corn which contains the transgenes. Corn line 6275 is a regulated article under APHIS regulations at 7 CFR Part 340 because some DNA sequences used to regulate the expression of these foreign genes in corn were derived from plant pests. The genes, along with these regulatory sequences, were introduced into the corn genome via using *Agrobacterium tumefaciens* strain LBA4404.

Field trials of 6275 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 an extension of a determination of non-

regulated status to 6275 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 line 6275 corn.

Corn line 6275 is similar to line 1507 (Table 1) and has been developed by Mycogen /Dow to provide farmers an alternative option for the control of larvae of certain lepidopteran insects which are significant pests in corn. A determination of nonregulated status was issued for corn line 1507, the antecedent organism, on June 14, 2001 (see 66 FR 42624 – 42625, August 14, 2001, Docket No. 00-070-3). The extension request from Mycogen/Dow for 6275 corn states that Bt Cry1F corn line 6275 is highly efficacious in the control of European corn borer (ECB), southwestern corn borer (SWCB), fall armyworm (FAW) and black cutworm (BCW), and moderately efficacious in the control of corn earworm (CEW). Larvae of ECB, SWCB, and FAW feed and burrow on corn leaves, stem whorls, stalks and/or ears resulting in stalk lodging, dropped ears, and damaged grain. BCW larvae cut off plants at or slightly below the soil surface, reducing plant stands. CEW feed primarily on the corn silk and ears resulting in yield loss and grain damage. *Bacillus thuringiensis* (Bt) bacteria produce a group of related toxins (delta-endotoxins) that when ingested by susceptible lepidopteran insects result in their death. Preparations of *B. thuringiensis* containing delta-endotoxins are used as foliarly applied biopesticides. However, they are not routinely effective against ECB and the other stalk boring larvae because at certain stages these larvae primarily feed inside the plants where the foliar applied biopesticide cannot reach. The same problem is encountered with other nonsystemic, foliarly applied chemical insecticides. The development and approval of transgenic corn plants expressing Bt delta-endotoxins has provided growers with another safe and efficacious option for the control of ECB which growers have widely embraced.

Corn line 6275 is also genetically engineered to express the enzyme phosphinothricin-N-acetyltransferase encoded by the *bar* gene derived from the bacterium *Streptomyces hygroscopicus*. (The petitioner refers to this enzyme as BAR to distinguish it from PAT which is encoded by the *pat* gene). Both PAT and BAR detoxify glufosinate and thereby confer tolerance to herbicides based on this active ingredient (e.g. the herbicides Basta®, Rely®, Finale®, and Liberty®). The herbicide tolerance provides an alternative weed management tool for farmers and a method of selecting for the corn which contains the transgenes. The truncated *cry1F* gene and the *bar* gene coding sequences were fused to regulatory sequences which enable the Cry1F and BAR proteins to be expressed constitutively throughout most of the plant. These regulatory regions were derived from genes from corn and from the plant pathogens cauliflower mosaic virus (CaMV) and *Agrobacterium tumefaciens*. No proteins are produced from the regulatory

regions themselves.

Agrobacterium tumefaciens-mediated transformation, a technique that is commonly used to introduce new genetic material into plants, was used to introduce these new gene constructs into corn to create the transgenic line 6275. Because line 6275 corn is engineered to contain genetic material from plant pathogens, it is considered to be a regulated article under APHIS regulations at 7 CFR Part 340.

Corn line 6275 has been field tested in a wide variety of locations (15 States and Puerto Rico) since 1999 under notifications or permits from APHIS that are listed in Appendix A. This field testing was conducted, in part, to confirm that line 6275 corn exhibits the desired agronomic characteristics and does not pose a plant pest risk. Although these field tests were conducted in agricultural settings, APHIS acknowledgment of notifications for the tests have stipulated that the regulated article and its offspring must not persist in the environment after completion of the test. Therefore, measures were employed to ensure physical and reproductive confinement from other sexually compatible plants and to manage volunteers. Reports for those field tests completed under APHIS authorization and other information contained in the petition have been submitted to APHIS upon which to base an extension to the determination of nonregulated status granted for corn line 1507 to corn line 6275.

Table 1. Genotypic Description of Corn Events DAS-01507-1 (line 1507) and DAS-06275-8 (line 6275).

Unique Identifier	**Event DAS-01507-1**[a]	**Event DAS-06275-8**
Crop	Corn	Corn
Cultivar Species name	*Zea mays* L.	*Zea mays* L.
Parent Line	Hi-II	Hi-II
Transformed Line	1507	6275
Event Designation	TC1057	6275
Transformation Method	Biolistic transformation	*Agrobacterium tumefaciens* mediated transformation
Vector	PHP8999	PHP12537
Trait	Lepidopteran-resistant, glufosinate-tolerant	Lepidopteran-resistant, glufosinate-tolerant
Gene 1/Donor	Plant-optimized (po) synthetic version of truncated *cry*1F gene from *Bacillus thuringiensis* var. *aizawai*. Two, linked copies of *cry*1F were inserted. The promoter for one of the two copies was truncated or absent.	Maize-optimized (mo) synthetic version of truncated *cry*1F gene from *Bacillus thuringiensis* var. *aizawai*.
Gene 1 Promoter/Donor	The ubiquitin 1 promoter (*ubi*ZM1) plus a ubiquitin 5' untranslated region and intron from *Zea mays*.[b]	Putatively, a truncation of the ubiquitin 1 promoter/UTR/intron (*ubi*ZM1(2)) element that contains a portion of the intron (starting from basepair +505[c]) from *Zea mays*.
Gene 1 Terminator/Donor	A terminator (ORF25PolyA) from *Agrobacterium tumefaciens* pTi15955.	Terminator (PINII) sequence from *Solanum tuberosum* proteinase inhibitor II.
Gene 2/Donor	Synthetic glufosinate tolerance gene (*pat*), based on a phosphinothricin acetyltransferase gene sequence from *Streptomyces viridochromogenes*.	Phosphinothricin acetyltransferase gene (*bar*) isolated from *Streptomyces hygroscopicus*.
Gene 2 Promoter/Donor	35S promoter Cauliflower Mosaic Virus.	35S promoter from Cauliflower Mosaic Virus strain 1841, and ADH1 intron from *Zea mays*
Gene 2 Terminator/Donor	35S terminator from Cauliflower Mosaic Virus.	Terminator sequence from *Solanum tuberosum* proteinase inhibitor II (PINII).

[a] DAS-01507-1 is the antecedent organism.

[b] Only one copy of the ubiquitin 1 promoter was detected, but it is not clear whether the probe used by DAS would have detected a promoter truncation similar to that found in line 6275. The probe used spanned base pairs 120-1707 and the promoter in line 6275 begins at approximately base pair 1552 in vector PHP8999.

[c] Numbering from Christensen et al. 1992, indicating the number of base pairs upstream of the second exon (the *cry*1F gene, in this case); this corresponds to base pair 1598 in the vector PHP12537.

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. Line 6275 corn has been considered a regulated article because some noncoding DNA regulatory sequences were derived from plant pathogens and a plant pathogen was used as the transformation vector.

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. According to 7 CFR Part 340.6(e), entitled “Extensions to determinations of nonregulated status”, APHIS may determine that a regulated article does not pose a plant pest risk and should not be regulated under 7 CFR 340 “based on the similarity of that organism to an antecedent organism.” A person may therefore request that APHIS extend a determination of nonregulated status to other regulated organisms and provide information that establishes the similarity of those organisms to the antecedent organism.

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

Line 6275 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 May 18, 2001, the EPA granted a conditional, time-limited registration for Cry1F as expressed in line 1507 in field corn that was scheduled to expire on midnight September 30, 2001. Subsequently, this registration was extended and will expire on October 15, 2008. Prior to this date, EPA will determine whether to extend the expiration date, convert the registration to a non-expiring registration, or let the registration expire. An application for registration of line 6275 corn has been submitted to the EPA and is currently being reviewed.

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; and the FDA enforces the tolerances set by the EPA. On June 15, 2000, the EPA announced receipt of the initial filing of a pesticide petition (PP 0G6112), submitted by Mycogen Seeds c/o Dow AgroSciences LLC, proposing an exemption from the requirement of a tolerance for residues of plant-pesticides Bt Cry1F protein and the genetic material necessary for the production of this protein in or on all food commodities (65 *FR* 37545-37547). On May 18, 2001 the EPA granted the proposed exemption for the Cry1F protein, but it is limited to field corn, sweet corn, and popcorn. The exemption 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. This exemption is applicable to all corn lines containing the Cry1F protein including 1507 and 6275.

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. Mycogen Seeds c/o Dow AgroSciences LLC and Pioneer Hi-Bred International, Inc. submitted a summary of their safety assessment of line 1507 to the FDA on June 28, 2000. On May 18, 2001 the FDA acknowledged the companies' conclusions that maize line 1507 is not materially different in composition, safety, or other relevant parameters from maize currently on the market, and that it does not raise issues that would require premarket review or approval of FDA, and they indicated that they had no further questions concerning line 1507. Data on line 6275 has been submitted to FDA (BNF0093) for comment.

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 line 6275 corn as a regulated article under APHIS regulations. Mycogen/Dow, the developer of line 6275 corn, submitted a request that the determination of non-regulated status granted to line 1507 corn be extended to corn transformation line 6275 and any progeny derived from crossing corn line 6275 with any other non-regulated corn varieties so that they would no longer be considered regulated articles under 7 CFR Part 340.

IV. ALTERNATIVES

A. No Action

Under the "no action alternative, APHIS would come to a determination that line 6275 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 line 6275 corn and its progeny.

B. Proposed Action: Extension of a Determination of Nonregulated Status to Line 6275

Under this alternative, line 6275 corn and its progeny would no longer be considered regulated articles under 7 CFR Part 340. Permits from or notifications to APHIS would no longer be required for introductions in the United States and its territories of line 6275 corn or its progeny. A basis for this determination would be that line 6275 corn does not pose a potential for plant pest risk based on its similarity to the antecedent organism line 1507 corn. Unrestricted cultivation of the line 6275 would be permitted by APHIS. Such a determination, however, does not preclude any restriction which might be placed on cultivation of this corn by other regulatory agencies also having authority over the use of this corn.

C. Determination of Nonregulated Status, in Part

The regulations at 7 CFR Part 340.6 (d) (3) (i) state that APHIS may approve 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.
In a decision to choose alternative A., no action, line 6275 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 line 6275 and any progeny derived from it.

If growers do not have improved varieties of corn seed derived from line 6275, they may choose to plant another cultivar with similar properties as an alternative such as the antecedent organism line 1507, or they may use other chemical or biological control mechanisms or management practices if they feel that their lepidopteran pest pressure and weed pressure is high enough to warrant it.

Other deregulated transgenic lepidopteran resistant corn expressing other Bt delta-endotoxins and other herbicide tolerant corn varieties are available by seed companies, and have been widely adopted by farmers in the United States (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). Other nontransgenic, corn borer-tolerant, hybrid varieties of corn are also available (Davidson and Lyons, 1987). Several chemical insecticides and biological or cultural control measures can be used to control the pests targeted by Bt Cry1F in line 6275 corn, and several herbicides and cultural practices can be used to manage weeds in corn. Details regarding the extent to which different control methods are currently employed, and the impacts from these are discussed in Section V. F. of this document.

No significant adverse impacts are envisioned if APHIS chooses alternative A.

Alternative B.
A decision to choose alternative B, an extension of deregulation to corn line 6275, is addressed below. The environmental impacts of unrestricted cultivation of corn line 6275 are compared to corn line 1507 and any impacts posed by the cultivation and distribution of corn not subject to APHIS regulation 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 corn line 6275 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 line 6275 corn.

Line 6275 was generated using plasmid PHP12537 (described in Figure 1 of the petition) via *Agrobacterium*-mediated transformation. Transformation with *Agrobacterium* should not lead to crown gall disease in 6275 because the *Agrobacterium tumefaciens* strain LBA4404 was disarmed by removing the native T-DNA from LBA4404, which contains the plant hormone genes necessary for the formation of crown gall tumors. Instead, a T-DNA region that contains the *cry1F* and *bar* gene coding sequences and the regulatory components necessary for their expression in the corn genome was introduced on a binary plasmid to create plasmid PHP12537 (described in Figure 2 of the petition). Further, antibiotics were used to kill any remaining *Agrobacterium* after transformation. The recipient corn line used in transformation line 6275 was the same public line designated Hi-II that was transformed to create line 1507 corn.

APHIS analyzed data that demonstrates that a corn plant regenerated from the embryo callus culture transformation, designated line 6275, contains one copy of the following genetic constructs derived from PHP12537 (See Table 1): (1) the truncated *cry1F* gene originally derived from *B.t.* var. *aizawai* strain PS811 whose transcription appears to be directed by a fragment of the first maize ubiquitin 1 (UBI1) intron and whose termination/polyadenylation sequences were derived from the *Solanum tuberosum* proteinase inhibitor II (PINII); and (2) the *bar* gene derived from *Streptomyces hygroscopicus* that encodes the enzyme phosphinothricin acetyltransferase (PAT or BAR) the transcription of which is driven by the cauliflower mosaic virus (CaMV) 35S RNA promoter fused with an alcohol dehydrogenase intron 1 (ADH1) from *Zea mays* and with a termination/polyadenylation sequence derived from the *Solanum tuberosum* proteinase inhibitor II. Data demonstrate that the BAR protein is expressed throughout the plant.

Because Southern analysis indicated that the promoter region of the *cry1F* gene in 6275 is truncated, the petitioner provided a sequence analysis of the truncated construct and the native maize DNA flanking the insertion site (see responses to letters of completeness that accompany the petition). Two lines of evidence suggest that there is not an endogenous maize promoter driving the expression of the *cry1F* gene. Transient expression assays by Salgueiro *et al.* (2000) suggests that some *ubi*1 intron constructs can direct gene expression in the absence of the *ubi*1 promoter region. While the truncated insert in 6275 is most similar to a construct that Salgueiro *et al.* found does not direct gene expression above background levels, the differences in expression systems (transient versus integrated, wheat versus maize) and the presence of a putative TATA box sequence within the intron, which is important for transcription initiation,

suggest that transcription could be directed by the truncated intron. Also, sequencing of 521 bases of maize DNA upstream of the truncated insert revealed no open reading frames of significant size or known promoter sequences. The 26-most upstream maize nucleotides sequenced were found to have perfect homology to a segment within the 5' untranslated region of a putative *Lactuca sativa* gene, called resistance gene candidate 2D (RGC2D), the function of which is not known but which is homologous to a gene that confers resistance in lettuce to a fungal pathogen (Meyers et al. 1998). The possibility remains that this sequence or further upstream sequences could be helping to increase expression of Cry1F; however, the absence of the rest of an RGC2D-like gene or another gene immediately adjacent to the truncated insert, suggests that an endogenous maize gene has not been interrupted and that endogenous active promoter and enhancer sequences are unlikely to be present. Furthermore, qualitative observations made by Dow Agro Sciences during field testing of 6275-containing hybrids indicated no change in disease susceptibility relative to the near isoline to fungal maize pathogens (See Petition, Section V.F.). Diseases that occurred were Northern corn leaf blight, Southern corn leaf blight, Southern rust, grey leaf spot, Stewart's wilt and smut. These observations suggest that the 6275 insertion does not effect resistance of the line to fungal pathogens. Despite the uncertain nature of the promoter for the *cry1F* gene, data demonstrate that the Cry1F protein is expressed throughout the plant (see Petition Section V.D. Tables 10 and 13 and Fig. 34).

The donor organisms for the *cry1F* and *bar* genes (*B.t.* var. *aizawai* strain PS811 and *Streptomyces hygroscopicus*, respectively) are soil-inhabiting bacteria. Neither of these bacteria are plant or human pathogens, and the Cry1F and BAR proteins encoded by these genes do not cause disease symptoms or the production of infectious agents in plants. The truncated *cry1F* gene coding sequences were modified for optimal expression in corn, in part, by changing its codon bias to that favored by corn. The protein encoded by the truncated *cry1F* gene is the same as that expressed in the antecedent organism line 1507. It is nearly identical to the first 605 amino acids of the Cry1F protein protoxin produced by the *B.t.* var. *aizawai* strain PS811. The only exception is a single amino acid substitution, leucine for phenylalanine at position 604. This truncated Cry1F protein corresponds to the insecticidally active portion of the delta endotoxin that remains following cleavage of the 569 amino acids from the end of the 1174 amino acid full length protoxin in the gut of susceptible lepidopteran larvae. The BAR protein catalyzes an acetylation reaction which converts L-phosphinothricin, the active ingredient in glufosinate ammonium herbicides, to an inactive form (OECD, 1999).

Some of the noncoding regulatory sequences that were fused to the *bar* gene to allow constitutive expression and processing of their messenger RNA (mRNA) in plants were derived from the CAMV plant pathogen. CaMV is a plant virus which causes disease primarily in cruciferous plants. The CAMV promoter does not cause disease symptoms in plants, nor does it encode the

production of an infectious agent.

The line 6275 corn plant was crossed with an elite inbred corn line to produce seed used for further breeding and analysis. APHIS analyzed data and information submitted in the petition that characterize the nature, stability, inheritance, and expression of the inserted genetic constructs and their encoded proteins in different generations of plants derived from line 6275.

DNA analysis of seeds from one generation (see Petition, Section V.A., Table 4 and Figs. 3-17) supports the conclusion that line 6275 contains within its genomic DNA (nuclear chromosomes) a single copy of an insertion containing the complete coding regions for *cry1F* with a truncated promoter region and intact terminator region and the complete coding regions for a *bar* gene with its associated noncoding regulatory regions. Additional DNA analysis of seeds from three different generations (see Petition, Section V.B., Tables 7-8 and Figs. 28-31) showed that these genetic constructs were stably inherited and cosegregated over five generations. As expected, data also show that sequences from outside of the right and left border sequences of PHP12537, including bacterial antibody resistance markers, are not present in line 6275. Data provided for eight generations (see Petition Section, V.C. Table 9) support the conclusion that the *bar* gene is stably inherited and dominant. Data collected on the eighth generation show that the glufosinate- and European corn borer-resistance traits are tightly linked.

Data characterizing the expression of the encoded Cry1F and BAR proteins in hybrids derived from line 6275 (see Petition Section V.D. Tables 10 and 13 and Fig. 34) support the conclusion that, as expected, the proteins are constitutively expressed and are of the correct molecular weight, with some minor proteolytic degradation of Cry1F. No proteins of higher molecular weight, which could represent fusion proteins, were detected. Cry1F and BAR proteins were detectable in plants collected at six different field sites over the growth of the plants (V9, R1, R4, maturity, senescence) in one or more tissues per life stage, including whole plant, roots, leaves, pollen, stalk, and grain. Efficacy data submitted with the petition support the conclusion that hybrids derived from line 6275 exhibit the expected trait conferred by the expression of the *cry1F* gene, i.e., resistance to lepidopteran insects (see Petition, Section V.E.1 Tables 22-23).

Reports evaluated by APHIS for field tests conducted with line 6275 since 1999 in 15 States (California, Hawaii, Illinois, Indiana, Iowa, Kentucky, Michigan, Missouri, Minnesota, Mississippi, Nebraska, North Dakota, Ohio, and Wisconsin) and in Puerto Rico indicate that no differences were observed between line 6275 hybrid corn and the non-transgenic hybrid counterparts for disease and pest susceptibility, other than resistance to the targeted lepidopteran pests. These tests included the major corn growing areas of the United States. Therefore, no unanticipated plant pest effects are expected to result from their cultivation. Corn derived from

line 6275 is not expected, nor has it been observed, to exhibit new disease symptoms or cause such symptoms to occur in other plants.

The Cry1F protein expressed in line 6275 is the same as the Cry1F protein expressed in line 1507, the antecedent organism. The Cry1F protein is expressed throughout the plant in both lines, but higher mean expression levels were observed in all plant tissues except for pollen in line 6275 compared to 1507 (Table 2). Data show that the genetic constructs were stably inherited and cosegregated over five generations for line 6275. Similar results were obtained for over four generations for line 1507. The efficacy of line 6275 is comparable to 1507 for ECB, SWCB, FAW, BCW, corn earworm (CEW; *Helicoverpa zea*) and western bean cutworm (WBCW; *Richia albicosta*).

Table 2. Comparison of Cry1F Tissue Expression in Plant Parts of Lines 6275 and 1507

		Line 6275			Line 1507		
Tissue	Growth Stage	Mean (ng/mg tissue dry wt)	Std Deviation	Min/Max (ng/mg tissue dry wt)	Mean (ng/mg tissue dry wt)	Std Deviation	Min/Max (ng/mg tissue dry wt)
Leaf	V9	16.7	4.6	0 - 23.8	12.1	6.2	0 - 24
Whole Plant	V9	6.22	1.16	4.98- 7.87	5.2	1.9	2.6 - 6.8
Whole Plant	R1	7.16	1.45	5.32 - 9.57	3.6	1.1	2.5 - 4.7
Pollen	R1	3.67	0.34	3.09 - 4.6	21.9	2.9	16.4 - 27.2
Stalk	R1	11	2.67	6.77 - 16.4	5.8	1.7	3.3 - 10.3
		Line 6275			Line 1507		
Forage	R4	6.26	1.09	5.05 - 7.77	1.7	1.1	0 - 3.2
Whole Plant	Senescence	2.47	0.41	1.95 - 3.07	1.6	0.6	0.9 - 2.4

B. Potential impacts based on the relative weediness of line 6275 corn

APHIS assessed whether line 6275 corn is any more likely to become a weed than the non-transgenic recipient corn line, or other corn currently cultivated, including the antecedent organism. The assessment encompasses a thorough consideration of the basic biology of corn and evaluation of unique characteristics of 6275 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).

Corn line 6275 exhibits no characteristics that would cause it to be more weedy than the parent corn line or line 1507. As noted above, reports from field trials in the United States indicate that no differences were observed between line 6275 hybrid corn and the non-transgenic hybrid counterparts for disease and pest susceptibility, other than resistance to the targeted lepidopteran pests, nor were differences observed which might increase the plants ability to compete or persist as a weed. APHIS evaluated data submitted in the petition that show both late and early hybrid maize lines derived from line 6275 and line 1507 had similar agronomic performance traits compared to each other and to non-transgenic isogenic hybrids grown in various field trials across the United States in 2002 (see Petition Section V.E.2, Tables 25 and 26). Traits evaluated include yield (bushels/acre adjusted for moisture), grain density (weight in pounds of a bushel of corn adjusted to 15.5% moisture), percent moisture at harvest, accumulated growing degree units to reach reproductive maturity (5% pollen shed and silking), percent stalk and root lodging, dropped ears, top integrity, stand count, emergence, plant vigor, plant height and ear height. The line 6275 hybrids had statistically significant higher yields (based on LSD at the 0.05 level) in one trial and better top integrity which the petitioners attribute to better control of ECB. Although some statistically significant differences were observed in some cases for maturity, stand counts, or emergence vigor, either the trends were not consistent nor were they of a magnitude that would lead to increased weediness in line 6275. Data were also provided that indicate that hybrids of line 6275 are comparable to other corn hybrids in seed germination characteristics (see Petition SectionV.E.2 Table 27). Therefore, data do not indicate that hybrids derived from line 6275 would be any more competitive or vigorous in their ability to germinate, establish or reproduce in different environments or have other characteristics that would increase their capacity to compete or persist as a weed.

The introduced genetic constructs and new traits, lepidopteran insect resistance and tolerance to glufosinate herbicides, are not expected to cause line 6275 corn to become a weed. None of the characteristics of weeds 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. Genetically engineered corn varieties with these traits have been widely grown in the United States since at least 1996. Line

6275 corn is still susceptible to other insect pests and diseases of corn.

In the United States, when corn fields are rotated to another crop, usually soybeans, corn plants may volunteer and pose a minor weed problem. Glufosinate-based herbicides are used for post-emergent control of many broadleaf and grassy weeds. Volunteers of line 6275 corn or offspring of crosses between line 6275 corn and other non-herbicide tolerant corn lines could be controlled using physical methods or with the use of other herbicides that are not based on glufosinate and which are registered for use on the crop, as appropriate. If crop varieties resistant to different herbicides are planted within pollination distance of each other (e.g. in adjacent fields), volunteers with multiple herbicide tolerance could emerge in the subsequent growing season. However, several factors minimize the likelihood of such occurrences in corn: (1) temporal differences in pollination dates and/or planting dates of different varieties will reduce the likelihood of concurrent periods of pollen shed or silking; (2) the pollen load within a given field will tend to swamp out the effect of pollen drift from adjacent fields; (3) corn pollen is only viable for up to 2 hours under optimal conditions (Herrero and Johnson, 1980; Luna *et al*, 2001); (4) corn pollen concentration drops off rapidly from the source to less than 1% within 60 meters (Raynor *et al.*, 1972); and (5) strict measures are taken to ensure genetic purity during production of hybrid corn seed. By making appropriate choices in varieties, planting locations, crop rotations, and herbicides, growers can minimize such occurrences. Despite the fact that corn varieties tolerant to the herbicides glufosinate-ammonium, glyphosate, imidazolinone, or sethoxydim have been planted over at least the last five years (at about three percent of U.S. corn acreage in 1996 to about 19 percent in 1998) (Fernandez-Cornejo and McBride, 2000), APHIS could find no reports of multiple herbicide tolerant corn volunteers posing a weed problem. Should such multiple herbicide tolerant volunteers occur with varieties developed from corn line 6275 and corn that is tolerant to another herbicide active ingredient with a different mode of action, an alternative herbicide with a mode of action different from those to which resistance has developed or other measures such as mechanical control can be used to control the volunteer if it poses a weed problem in a subsequent crop.

APHIS concludes that, with the exception of increased resistance to certain lepidopteran insects and tolerance to glufosinate herbicides, line 6275 corn has agronomic traits similar to those of traditionally bred corn and line 1507, and it does not exhibit traits that would cause increased weediness. Its cultivation should not lead to increased weediness of other cultivated corn.

C. Potential impacts from gene introgression from line 6275 corn into its sexually-compatible relatives.

APHIS evaluated the potential for gene introgression to occur from line 6275 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 to varying degrees with other members of the genus *Zea* collectively referred to as teosinte and to a much lesser extent with members of the genus *Tripsacum* (see Appendix B for a more detailed discussion).

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 American teosinte 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 F1 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* 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). 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 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 6275 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 use of import grain for planting. Since 6275 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 6275 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 a weeds.

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

APHIS evaluated the potential for line 6275 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 expression of BAR 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 BAR or PAT proteins. The DNA encoding the BAR protein is not toxic and the BAR protein shares no significant homology with proteins known to be toxic or allergenic (OECD, 1999). Additional information was provided in Appendix II of the petition (Korjagin, 2003, Song, 2002) to support that the BAR protein does not have characteristics commonly attributed to allergenic proteins.

The Cry1F protein expressed in line 6275 corn is similar to the well known Cry1A class of lepidopteran-specific toxins produced by Bt strains. The specificity of the insecticidal activity of these Cry proteins appears to be dependent upon their binding to specific receptors present in the mid-gut of lepidopteran insects (Lambert, *et al.*, 1996; Van Rie *et al.*, 1990; Van Rie *et al.*, 1989; Hofmann *et al.*, 1988a and 1988b; and Wolfersberger *et al.*, 1986). These insecticidal proteins are not expected to adversely effect other invertebrates and all vertebrate organisms, including non-target birds, mammals and humans, because these organisms would not be expected to contain the receptor protein found in the insect's midgut. APHIS evaluated laboratory and field

studies on representative species that support these expectations.

Potential impacts on nontarget, non-lepidopteran pests.
Target pests of the modified Cry1F protein expressed in line 6275 corn are larvae of certain lepidopteran pests of corn. Field test reports for APHIS notifications and efficacy studies submitted indicate that, as expected, corn line 6275 hybrids are protected to varying degrees against feeding damage from certain lepidopteran pests including ECB, SWCB, FAW, BCW, WBCW, CEW, and the level of protection was not significantly different compared to line 1507 hybrids (See Petition, Section V.E.1). The petition notes that breeders visually monitored the *B.t.* Cry1F corn line 6275 hybrids and non-modified maize lines during field tests conducted under APHIS notifications for pest resistance, and they reported no differences in insect damage caused by non-lepidopteran pests such as thrips, aphids and red spider mites.

Potential impacts on non-target organisms, including beneficial organisms.
APHIS previously evaluated the results of several studies submitted in support of deregulation of the antecedent organism that were designed to evaluate the sensitivity of representative non-target organisms to Cry1F as expressed in different test substrates: i.e., corn grain or pollen expressing plant-optimized Cry1F protein from line 1507 corn; or Cry1F purified from a *Pseudomonas fluorescens* bacterial strain engineered to express the protein toxin. The results of these studies are summarized in Appendix C of this Environmental Assessment. APHIS concluded that the petitioner adequately demonstrated that the bacterially-produced Cry1F, as purified and prepared for these studies, was similar enough in its biochemical properties (molecular weight, amino acid sequence, and lack of glycosylation) and in its biological activity against lepidopteran larvae to warrant its use as a test substance comparable to Cry1F as produced in line 1507 corn. In both cases, the predominant active protein purified from these sources was a protease-resistant core protein with a molecular weight of approximately 65 kDa. Because the Cry1F protein expressed in line 6275 corn is the same as that expressed in line 1507 corn, these data are applicable for the extension to 6275 corn. Tests included acute dietary toxicity studies with beneficial arthropods such as honey bee larvae, predatory lady beetle (*Hippodamia convergens*) and green lacewing (*Chrysoperla carnea*) and parasitic Hymenoptera (*Nasonia vitripennis*); a 28 day chronic effects study on survival and reproduction of the soil-dwelling arthropod Collembola (springtails; *Folsomia candida*); and acute toxicity studies with other non-target organisms including earthworms, the freshwater invertebrate *Daphnia magna*, Northern bobwhite quail, and mice. Results of these studies indicate that no deleterious effects on these organisms would be expected due to incidental exposure or feeding on line 1507 corn. This analysis took into consideration the levels of the Cry1F protein measured in different tissues of line 1507 corn, the environmental fate and likely routes and levels of exposure to line 1507 corn plant tissue or residues of this tissue that contain the active toxin, and dietary preferences. Since the Cry1F protein measured in pollen and seeds of line 6275 corn were 1/6 and ½ the

respective concentration in the respective tissues in line 1507 corn, exposure to Cry1F via pollen to pollinators, beneficial arthropods, and freshwater species, and via grain to avian species, and grain feeding rodents and other mammals would be even less than for line 1507 corn. Soil exposure to Cry1F from line 6275 is expected to be 1.5 times greater than for line 1507 based on differences in levels measured in whole plants during senescence (see Table 2 of this EA). But since the estimated LC_{50} for Cry1F from line 1507 for Collembola and earthworms was at least 198 fold and 40 fold, respectively, than the estimated environmental soil concentration of Cry1F from line 1507, there is still expected to be a sufficient margin of safety for these soil-dwelling organisms from Cry1F arising from line 6275.

In addition to the laboratory studies, results of a small scale field study conducted with line 1507 in 1999 in Johnston, Iowa demonstrated that there was no consistent pattern of differences in abundance of several categories of beneficial arthropod predators observed in plots planted to Bt. Cry1F or the non-transformed genetically similar corn. Another study with 1507 corn planted in Sheldahl, IA in 2001 had similar results. Although no non-target insect field studies have been submitted with corn line 6275, similar results are expected since the Cry1F protein is the same as in line 1507.

Potential impacts on monarch butterflies

A 1999 study by Losey *et al.* reported that pollen from a certain line of Bt corn was harmful to monarch butterfly larvae when dusted onto milkweed leaves under laboratory conditions at a single concentration. The study was highly limited in its usefulness, as it did not account for the many variables that affect monarch butterfly populations under natural conditions. For example, corn pollen is heavy and does not travel long distances from its source in significant amounts and is diluted as it moves. Within the corn fields, milkweeds are controlled by farmers as a part of their routine weed control practices. The EPA has concluded that the other two most widely planted varieties for corn borer control would not be deposited on milkweed plant with toxic amounts. The risk of a significant impact on monarch populations from Bt corn is therefore very low. This conclusion is consistent with the findings of several scientists that were published as several reports in the Proceedings of the National Academy of Sciences (PNAS) and summarized in an accompanying risk assessment by Sears et al. (2001).

The toxicity to monarch larvae of Cry1F as expressed in lines 1507 and 6275 is even lower than the currently registered Cry1Ab Bt corn plant-incorporated protectants (PIPs). The expression of Bt Cry1F is much lower in 6275 corn pollen (3.67±0.34 ng/mg tissue dry wt.) than in 1507 (21.9±2.9 ng/mg tissue dry wt.). The EEC of Cry1F on milkweed leaves due to surface deposits of pollen from hybrids of Bt Cry1F line 1507 is estimated to be less than the LC_{50} (that concentration at which 50% mortality is observed) for greater than 90% of lepidopteran species at distances greater than 0.2 m from the field edge. This estimate is probably representative of

what one would expect for pollen disposition on other weeds or plants growing in and around corn fields. Therefore, cultivation of line 6275 corn is not expected to harm monarch butterfly larvae nor is it expected to significantly affect the majority of other non-target lepidopteran larvae beyond the field margins.

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 Cry1F activity, species outside the insect order Lepidoptera should also not be affected. EPA has thoroughly examined all threatened and endangered lepidopterans that occur in counties where corn is grown, and determined that the breeding habitat of lepidopterans does not overlap corn.

APHIS examined threatened and endangered species as part of the assessment for 1507 corn (Petition 00-136-01p) which is also applicable to 6275 corn. Because of the lack of toxicity of the BAR protein and the demonstrated toxicity of Cry1F to only certain species of lepidopteran larvae, APHIS focused its analysis of impacts on threatened and endangered lepidopteran species. A Biological Opinion from the Department of Interior Fish and Wildlife Service was issued on December 18, 1986, concerning possible effects of foliar spray of *B. t.* subsp. *kurstaki* on threatened and endangered species. Based on difference in exposure routes between foliar spray and expression in plants, APHIS believes that the Biological Opinion is inapplicable. The majority of endangered lepidopterans in the U.S. have very restrictive habitat ranges; and their larvae typically feed on specific host plants, none of which include corn or its sexually compatible relatives. An examination of county distribution of endangered lepidopterans shows that, for the most part, they do not occur in agricultural settings where corn is grown. The only possible exceptions are Karner blue butterfly and Mitchell's satyr butterfly. APHIS previously examined the potential for impact on these two species due to exposure to corn pollen expressing Cry1F landing on their host plants. The assessment of risk to monarch butterfly associated with non-target exposure to maize pollen containing Cry1F on their milkweed host plant indicates rapid fall-off in exposure with distance, and consequently there is limited potential for non-target effects beyond the immediate field extremity (Pleasants *et al.* 2001).

Mitchell's satyr butterfly occur in northern wetlands fed by seeps and springs known as fens, and their larvae, which are present throughout the summer, feed primarily on sedges (USFWS, 1999). Some of the populations have been observed within 800 meters of corn fields (Wayne Wheling, APHIS Entomologist, personal communication to Susan Koehler). This distance should be sufficient to preclude exposure to toxic concentrations of pollen containing Cry1F. Pollen drift onto sedges in these fields will be further inhibited by the pines and oaks that typically surround these habitats.

The Karner blue requires wild lupine *(Lupinus perennis)* as an oviposition substrate and larval food source, while the adults feed on wild flowers. As of 1992, Karner blue is known to exist along the northern extent of the range of wild lupine, where there are prolonged periods of winter snowpack, in parts of Wisconsin, Michigan, Minnesota, Indiana, New Hampshire, New York, and Illinois (Haack, 1993). Karner blue is associated with wild lupine growing on dry, sandy soils in pine barrens, oak savannah, forest trails and previously disturbed habitats such as utility rights-of-way, military installations, airports, highway corridors, sand roads and sand pits, and abandoned farm fields (Haack, 1993). Wild lupine thrives in full sun to partial shade, and does not survive long in full shade (Haack, 1993), and thus would not survive long in a mature corn field. Likewise, the Karner blue is associated with areas of low to semi-closed canopy cover (Haack, 1993). Therefore, Karner blue larvae are not expected to be found in a mature corn field. In an addendum to their Environmental Assessment for the pesticide registration for Cry1F line 1507 corn dated April 27, 2001, the EPA indicated that "there are anecdotal reports of wild lupine growing 'within a couple of hundred meters of corn fields'" and that "there are recent reports that wild lupine may, in rare instances, grow in the vicinity of corn fields, especially in cases where the field may have been fallow in the previous season". They noted however, that "there are no reports of Karner blue larvae or wild lupine within one meter of corn fields."

Furthermore, as assessed in the EA for line 1507, the overlap of Karner blue larval feeding with the period of corn pollen shed is very unlikely because only second generation larvae have any potential for overlap with the shed of corn pollen in those areas where both occur and the bulk of pollen shed occurs after larval feeding ceases. Because Cry1F protein is active against Lepidoptera, some activity against the Karner blue at high dose levels would not be surprising, particularly for the younger larvae should they be exposed, which is unlikely. While the NOEL (no observable effect level) for Karner blue larvae has not been determined, it is unlikely, based on data from other lepidopteran larvae that effects would be observed at distances greater than 1 meter from the field margin.

Based on this analysis, APHIS concludes that cultivation of line 6275 corn should not have a significant potential to harm non-target and beneficial organisms common to agricultural ecosystems, nor will it effect species recognized as threatened or endangered by the U.S. Fish and Wildlife Service.

E. Potential impacts on biodiversity

Our analysis concludes that line 6275 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 lepidopteran-resistant and glufosinate-ammonium tolerant corn line 6275 on current agricultural practices, in particular, those used to control lepidopteran insect pests and weeds in corn and other crops. The potential impacts on organic farming were also considered. The impacts are not expected to be different than those previously analyzed for line 1507.

Impacts of previously deregulated lepidopteran-resistant corn on insect control
To examine the potential impacts of cultivation of Bt Cry1F line 6275 corn, APHIS considered the impacts that other lepidopteran resistant Bt corn varieties have had on agricultural practices in the U.S. The major pest controlled by these Bt corn varieties is the ECB, but other important pests controlled to varying degrees are CEW, SWCB, and other stalk boring lepidoperan larvae. A risk and benefits assessment for reregistration of Bt corn and cotton PIPs has been prepared by the EPA (U.S. EPA, 2000a) and is posted at the following EPA internet site: *http://www.epa.gov/scipoly/sap*. Issues being considered by the EPA pertaining to this assessment were the subject of a meeting convened on October 18-20, 2000 by the EPA Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and Food Quality Protection Act (FQPA) Scientific Advisory Panel (SAP). 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 this new technology. Estimates of Bt corn acreage as a percent of total corn acreage planted are 1% in 1996 to 26% in 1999, 2000, and 2002 (USDA NASS, and http://www.usda.gov/nass/pubs/bioc0703.pdf). EPA's analysis of pesticide usage in corn for the major corn-producing states for which data were available shows that for insecticides recommended for ECB control, acre treatments with respect to acres planted have declined from 8% in the 3 years prior to the introduction of Bt corn (1992 to 1995) to 5% in 1999. The four states with highest percentage of Bt corn (25 to 36%) saw a reduction from 6 million to slightly over 4 million (about one-third) in the number of acre treatments of insecticides recommended for ECB control. Most of the reduction has been with the organophosphate insecticides chlorpyrifos and methyl parathion, which are also registered for control of corn rootworm (CRW) larvae and/or adults. Total corn insecticide usage did not show a decline, perhaps because the 4 high adopter states are also high CRW states. Most of the insecticide used in corn in the major corn producing states in the midwestern cornbelt in 1996

was targeted at CRW control (Fernandez-Cornejo and Jans, 1999, Appendix 1, Table 1.1). The same is true for 1998, as compiled statistics on corn insecticide use across 16 major corn producing states indicate that chemical insecticides registered for CRW control were applied on over 33% of this corn acreage (USDA, 1999).

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. The IRM plan that is currently being used for commercial Bt corn lines was developed by the National Corn Growers Association in cooperation with biotechnology providers and university entomologists. The plan includes monitoring for compliance with the IRM plan, monitoring for the development of resistant ECB, SWCB, and CEW populations, and mitigation measures in the line that resistant populations are confirmed. Bt Cry1Ab corn and Bt Cry1Ac cotton, have been in commercial production since 1996 and Bt Cry1F corn line 1507 has been registered by the EPA for commercial production since 2001. There has been no reported lepidopteran insect resistance to the Bt toxins expressed in corn (U.S. EPA, 2000a, Tabashnik *et al*. 2003). For corn, this includes ECB, CEW, and SWCB.

Potential impacts of line 6275 corn on insect control practices

Efficacy data from field evaluations conducted in 2002 in a number of major corn-growing states (Petition, Section V.E.1) indicate that Cry1F line 6275 corn is statistically more efficacious than the comparable non-Bt isogenic hybrid corn in the control of ECB, SWCB, FAW, WBCW, and BCW and moderately efficacious in the control of CEW, and it can provide significantly superior control of FAW and BCW and equal or slighter greater control of ECB, SWCB, and CEW than currently marketed *Bt* Cry1A transgenic lines. The level of protection of hybrids containing line 6275 was similar to the protection level provided by the previously deregulated Cry1F line 1507. Therefore, growers may choose to adopt Bt Cry1F corn line 6275 instead of non-transgenic corn or the current transgenic lepidopteran-resistant Bt corn lines, particularly if they experience heavy pest pressure from FAW and BCW.

Data from 1996 for 16 states surveyed indicate that of the total acre-treatments of insecticides, 62% were targeted at CRW, 11% were targeted at cutworms and armyworms, and 19% were targeted at other moths and caterpillars (including cornborers) (Fernandes-Cornejo and Jans, 1999; Petition 00-136-01p, Appendix 1, Table 1.1). At least 6 of the 16 states used a higher percentage of pesticides on cutworms and armyworms than on other caterpillars and moths. These include Indiana, Kentucky, Missouri, Ohio, Pennsylvania, and South Carolina. Since none of these states are among the highest adopters of Bt corn (U.S. EPA, 2000a), there may be new markets for 6275 corn as with line 1507 in those states. Based on this analysis, APHIS believes that cultivation of Bt Cry1F corn line 6275 has the potential to further reduce chemical

insecticide applications targeted not only for ECB and SWCB, but for cutworms and armyworms as well, provided these insecticides are not also being applied to control other pests such as CRW. Chemical control options for cutworms and armyworms include planting-time soil insecticide applications (primarily organophosphates, carbamates, or phenylpyrazoles or rescue insecticide applications (primarily pyrethroids). Some of the chemical insecticides recommended for the control of ECB and SWCB include carbamates (carbofuran and carbaryl), organophosphates (e.g.,chlorpyrifos, methyl parathion) and synthetic pyrethroids (e.g., permethrin, lambda-cyhalothrin, and esfenvalerate) (Mississippi State University Extension Service, 1999; Gray and Steffey, 1999). Because many of these insecticides also kill predators or parasites that help to keep minor pests under control, additional pesticides are sometimes applied to kill mites and/or sucking insects (e.g. dimethoate). Many of these insecticides are more toxic to humans and non-target organisms (including some of the natural parasites or predators used to control them) than are Bt delta endotoxins (for example, see Petition 00-136-01p Appendix C; and Gray and Steffey, 1999); therefore, a reduction in their use should provide benefits to the environment as well as to humans, particularly farm workers and their children who are at higher risk from exposure.

As for line 1507, APHIS also does not anticipate that cultivation of line 6275 corn would affect the use of other biological or cultural control methods for the target pests since these methods are used on less than about 3% of the total corn acreage, particularly by organic farmers. Survey data from 1996 on pest management practices in corn indicate that Bt foliar insecticides were used on only 2.4% of insecticide-treated acres, and beneficial insects were released on less than 0.5 % of acres planted (Fernandez-Cornejo and Jans, 1999, Petition 00-136-01p, Table 8). This is despite the fact that several Bt foliar insecticides (based on *B.t. kurstaki* and *B.t. aizawai*) and beneficial insects such as the tachinid fly *Lydella thompsoni*, *Trichogamma* parasites and spined soldier bug (*Podisus maculiventis*), as well as other biologicals, such as the fungus *Beauveria bassiana,* are available for control of the same pests targeted for control in corn line 6275 (see *http:www.agrobiologicals.com*).

As with line 1507, line 6275 corn could also provide a similar tool for managing target insects that might become resistant to other insecticides currently used, including potentially other Bt-based insecticides. The IRM plan submitted for Bt Cry1F corn line 6275 is the same IRM plan that is currently being used for the other commercial Bt corn lines including Cry1F line 1507. The pesticide registration for all Bt corn lines specifies that growers will be required to sign a Stewardship Agreement affirming their intention to comply with the IRM requirement. Therefore, APHIS does not expect this will result in a significant change in agricultural practices. With the intensive monitoring programs in place for all Bt PIPs, Bt toxin resistant insect populations, should they develop, are likely to be detected and mitigation actions put in place as called for in the IRM plans and/or the registration conditions.

APHIS concludes that cultivation of line 6275 corn should pose no greater impediments on the control of insects in corn and other crops than the currently practiced methods of control of the target pests, ECB, SWCB, FAW, and BCW; i.e., the use of ECB-tolerant corn cultivars, including other previously deregulated Bt transgenic corn transformation lines, including line 1507 and the application of chemical and biologically-based insecticides.

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% preemergence, 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 (including Liberty Link® varieties) was associated with an overall decrease in herbicide use in 1996 (especially for the chloroacetamide herbicide family) (Fernandez-Cornejo and Klotz-Ingram 1998). Nonetheless, in 1997, 96% of the corn acreage in the 10 major corn-producing states were treated with herbicides. At least 18 different herbicide active ingredients are 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 account for 72% of the total applied in 1997 (Knake 1998; Fernandez-Cornejo and McBride 2000). In 1998, it is estimated that 18.4% of corn acreage planted was to herbicide-resistant varieties (some of which are stacked with *Bt cry* genes) (Fernandes-Cornejo and McBride 2000) and 7.5% of corn acreage was planted to Liberty Link® corn (Carpenter and Gianessi 1999).

Potential impacts of line 6275 corn on weed control

APHIS evaluated data submitted by the petitioners that show that hybrids derived from line 1507 corn exhibit tolerance to glufosinate ammonium herbicides at concentrations that provide effective weed control and excellent crop safety (see Petitioners response to Petition 00-136-01p deficiency number 3 dated July 19, 2000). Liberty glufosinate-ammonium herbicide is currently registered by the EPA for use only on Liberty Link® (glufosinate-ammonium tolerant) crops - field corn, soybeans, sugarbeet, canola, and on potatoes for desiccation only. Line 6275 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 and residually active herbicides such as atrazine; 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 6275 corn, like line 1507 corn, can be easily 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 6275 corn in Roundup Ready soybeans. It is estimated that in 1998, 26% of the total soybean acreage was planted to Roundup Ready soybeans and 54% (approximately 40 million acres of the 75.4 million acres of the soybeans grown in the United States) was Roundup Ready in 2000 (Carpenter and Gianessi, 1999, http://www.agbios.com/dbase.php). 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 6275 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 six weed species (as of May 20, 2004) 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. APHIS and the EPA Herbicide Division 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) non-transgenic 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 and labeled as Bt Cry1F lepidopteran resistant, and based on the IRM plan, farmers will be educated about recommended management practices. Glufosinate tolerant and lepidopteran resistant Bt corn, including line 1507, is already being used by farmers. Line 6275 corn will in some cases be used by some farmers instead of the existing lines, but should not present new and different issues. 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 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 low, even if flowering times coincide. The frequency of such an occurrence decreases with increasing distance from the pollen source such that it is sufficiently low by 660 feet away, the isolation distance considered acceptable 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 significant differences between 6275 and non-transgenic hybrid counterparts that would be expected to cause a either a direct or indirect plant pest effect on any raw or processed plant commodity from an extension of deregulation to line 6275 (Petition Tables 16-18).

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 futures 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 6275 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) to predict the cumulative impacts of deregulation and any subsequent registration and commercialization of another Cry1F corn, in light of other transgenic lepidopteran-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 nontarget pests, and the potential for resistance to the Bt toxins to develop as a result of exposure to these toxins in Bt PIPs or in other Bt formulations. Because of the uncertain possibility for target pests to develop cross-resistance to Cry1F and Cry1Ab Bt proteins, researchers and the EPA do not recommend, nor do companies intend to develop hybrids with combinations of these genes.

From this analysis, we are reasonably certain that no significant cumulative impact would result if our proposed action, an extension of deregulation to corn line 6275, is taken. Given current agricultural, breeding, and regulatory practices or requirements, any potential adverse effects that can reasonably be predicted are likely to be prevented, and if not at least detected and mitigated before a significant impact could occur. As described in Section II, even if a determination of non-regulated status is granted to corn line 6275, cultivation of this line or its progeny would still be limited under regulations by the EPA that require an experimental use permit for pesticides until they are registered conditionally or unconditionally for seed increase or full commercial use, and feed and food use would be regulated by the EPA and FDA.

Alternative C, Approval of the Petition in Part

Approval of some but not all of lines requested in the extension request. The petition requested an extension of the determination of nonregulated status only for line 6275 derived from the one transformation event, designated as TC6275. Therefore, APHIS can consider only that one line for approval.

Approval of the petition with geographic restrictions. EPA is currently reviewing the application to register 6275 corn as a plant pesticide. EPA has completed a thorough analysis of risks to non-target organisms and to threatened and endangered species. After examining all threatened and endangered lepidopterans that occur in counties where corn is grown, they have concluded that none of the lepidopteran breeding habitats are shared with corn. Based on this finding, APHIS finds no reason to place geographic restriction on planting of line 6275 corn.

VI. CONSIDERATION OF EXECUTIVE ORDERS, STANDARDS AND TREATIES RELATING TO ENVIRONMENTAL IMPACTS

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 benefitting 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 BAR, establishes the safety of corn line 6275 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 an extension of deregulation to line 6275 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 extended to line 6275 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 6275 corn subsequent to an extension of non-regulated status to line 6275 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. 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 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 82 countries are parties

to it as of Jan. 21, 2004 (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 asessment 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 developed 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 regularly in biotechnology policy discussions at forums sponsored by the European Union and periodically holds bilateral discussions on biotechnology regulatory issues with other countries (e.g. with Canada, Mexico, Argentina, Brazil, Japan, China, Korea to name a few). Mexico, which has relatives of corn that can potentially interbreed with it, has procedures in place that require a full evaluation of transgenic plants before they can be introduced into the environment and has ratified the Cartagena Protocol. 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).

VII. CONCLUSIONS

This environmental assessment addresses questions pertinent to the risk to the human environment, including plant pest risks, that could potentially result from an APHIS determination of nonregulated status under 7 CFR Part 340.6 for corn line 6275 and its progeny and their subsequent cultivation in the United States and its territories. It also considers restrictions placed on the cultivation of this line stipulated in the pesticide registration granted by the EPA. APHIS

has evaluated information from the scientific literature as well as data submitted in the petition that characterized line 6275 corn and progeny derived from it. After careful analysis, APHIS has come to the following conclusions:

1. Line 6275 corn exhibits no plant pathogenic properties. Although DNA from pathogens were used in its development, these plants are not infected by these organisms, nor can these plants incite disease in other plants.

2. Line 6275 corn is no more likely to become a weed than insect or herbicide tolerant corn that is currently being cultivated. Corn is not a weed, and there is no reason to believe that the introduced genes would enable corn to become a weed pest.

3. Introgression from line 6275 corn into wild plants in the United States and its territories is extremely unlikely. Potential introgression from line 6275 corn into wild relatives is not likely to increase the weediness potential of any resulting progeny nor adversely effect genetic diversity of related plants any more than would introgression from traditional corn hybrids.

4. Line 6275 corn is substantially equivalent in whole plant forage composition and in kernel composition, quality and other characteristics to nontransgenic corn and should have no adverse impact on raw or processed agricultural commodities.

5. Line 6275 corn will not have a significant adverse impact on nontarget organisms, including those beneficial to agriculture; and it will not affect threatened or endangered species.

6. Compared to current agricultural practices, cultivation of line 6275 corn should not reduce the ability to control insects or weeds in corn or other crops.

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IX. PREPARERS AND REVIEWERS

Biotechnology Regulatory Services Staff
Susan Koehler, Ph.D., Chief, Ecological and Environmental Assessment Branch
James L. White, Ph.D., Chief, Risk Assessment Branch
Robyn I. Rose, Biotechnologist/Ecologist (Preparer)
Laura Bartley, Ph.D., AAAS Risk Policy Fellow, Reviewer
Rebecca Stankiewicz-Gabel, Regulatory Analyst, Reviewer
Shirley Ingebritsen, Regulatory Analyst, Reviewer

X. AGENCY CONTACT

Ms. Terry Hampton, Regulatory Analyst
USDA, APHIS, Biotechnology Regulatory Services
4700 River Road, Unit 147
Riverdale, MD 20737-1237

Phone: (301) 734-5715
Fax: (301) 734-8669
terry.a.hampton@aphis.usda.gov

Appendix A: USDA Approved Field Tests of Bt Cry1F Corn Line 6275 Listed by n Notification Number.

03-052-08n
03-031-04n
03-031-03n
02-077-06n
02-060-17n
02-060-16n
02-060-15n
02-060-14n
01-116-07n
01-092-18n
01-092-16n
01-047-21n
00-224-02n
00-097-03n
00-083-03n
00-097-02n
99-357-08n
99-274-10n
99-078-10n
98-296-07n
98-288-18n

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

A few isolated populations of annual and perennial teosinte have been reported to exist in 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. 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 teosintes, *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 (KatoY., 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 6275 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 Cry1F (as expressed in corn Line 1507 or as purified from a microbial source) compared to other common insecticides used on corn to control the target pests European cornborer, southwestern cornborer, fall armyworm, black cutworm, corn earworm, and other non-target pests.

(Dimethoate is used to control nontarget pests including for example, corn leaf aphids, corn rootworm, grasshoppers, and spider mites. The other insecticides control one or more of the target pests. Insecticides were chosen based on a number of factors including a past history of moderate to high usage based on National Agricultural Statistical Service data for 1996 and 1998 and availability of safety data.)

	Cry1F [1] [Bt protein]	Dimethoate[2] (Cygon7) [organophosphate]	Chlorpyrifos[3] (Lorsban 7) [organophosphate]	Permethrin[4] (Ambush/Pounce7) [pyrethroid]
Environmental Fate	Cry1F protein is expressed in minute quantities and is retained within the plant. Therefore, common modes of toxicity or routes of exposure are generally not relevant to consideration of the cumulative exposure to *Bacillus thuringiensis* Cry1F insect control protein. The product has demonstrated low toxicity to a large number of organisms listed in this table. In addition, the protein is not likely to be present in drinking water because the protein is deployed in minute quantities within the plant. The time-dependent loss in bioavailability of CrylF protein following incorporation into a typical maize-growing soil was determined under laboratory conditions (Halliday, 1998). The results of this study indicated that soil-applied CrylF protein exhibited a greater than 20-fold decline in biological activity over the 28-day test period. The estimated DT_{50} was 3.13 days. These results are consistent with those for CrylAb protein using essentially the same experimental design; a soil DT_{50} of 1.6 days was reported for the CrylAb protein.	Dimethoate is of low persistence in the soil environment. Soil half-lives of 4 to 16 days, or as high as 122 days have been reported, but a representative value may be on the order of 20 days. Because it is rapidly broken down by soil microorganisms, it will be broken down faster in moist soils. Dimethoate is highly soluble in water, and it adsorbs only very weakly to soil particles so it may be subject to considerable leaching. However, it is degraded by hydrolysis, especially in alkaline soils, and evaporates from dry soil surfaces. Losses due to evaporation of 23 to 40% of applied dimethoate have been reported. Biodegradation may be significant, with a 77% loss reported in a nonsterile clay loam soil after 2 weeks. In water, dimethoate is not expected to adsorb to sediments or suspended particles, nor to bioaccumulate in aquatic organisms. The half-life for dimethoate in raw river water was 8 days, with disappearance possibly due to microbial action or chemical degradation.	In soils: Chlorpyrifos is moderately persistent with a half-life of usually 60 and 120 days, and a range from 2 wks - > 1 yr., depending on the soil type, climate, and other conditions . It was less persistent in soils with a higher pH (greater than 7.4). Soil half-life was not affected by soil texture or organic matter content. Adsorbed chlorpyrifos is subject to degradation by UV light, chemical hydrolysis and by soil microbes. When applied to moist soils, the volatility half-life was 45 to 163 hours, with 62 to 89% of the applied chlorpyrifos remaining on the soil after 36 hours. In another study, 2.6 and 9.3% of the chlorpyrifos applied to sand or silt loam soil remained after 30 days . Chlorpyrifos adsorbs strongly to soil particles and it is not readily soluble in water. It is therefore immobile in soils and unlikely to leach or to contaminate groundwater. TCP, the principal metabolite of chlorpyrifos, is moderately mobile and persistent in soils. In water: The concentration and persistence of chlorpyrifos will vary depending on the type of formulation. The increase in the concentration of	Permethrin is of low to moderate persistence in the soil environment, with reported half-lives of 30 to 38 days. Permethrin is readily broken down, or degraded, in most soils except organic types. Soil microorganisms play a large role in the degradation of permethrin in the soil. The addition of nutrients to soil may increase the degradation of permethrin. It has been observed that the availability of sodium and phosphorous decreases when permethrin is added to the soil. Permethrin is tightly bound by soils, especially by organic matter. Very little leaching of permethrin has been reported. It is not very mobile in a wide range of soil types. Because permethrin binds very strongly to soil particles and is nearly insoluble in water, it is not expected to leach or to contaminate groundwater. The results of one study near estuarine areas showed that permethrin had a half-life of less than 2.5 days. When exposed to sunlight, the half-life was 4.6 days. Permethrin degrades rapidly in water, although it can persist in sediments.

			insecticide is slower for granules and controlled release formulations in the water, but the resulting concentration persists longer . Volatilization is probably the primary route of loss of chlorpyrifos from water. Volatility half-lives of 3.5 and 20 days have been estimated for pond water. The photolysis half-life is 3 to 4 weeks during midsummer in the U.S. Research suggests that in water the rate at which it is hydrolyzed decreases by 2.5- to 3-fold with each 10 C drop in temperature. The rate of hydrolysis increases in alkaline waters. In water at pH 7.0 and 25 C, it had a half-life of 35 to 78 days. In vegetation: Chlorpyrifos may be toxic to some plants. Residues remain on plant surfaces for ~ 10 to 14 days. This insecticide and its soil metabolites can accumulate in certain crops.	Breakdown in vegetation: Permethrin is not phytotoxic, or poisonous, to most plants when it is used as directed. No incompatibility has been observed with permethrin on cultivated plants.
Avian toxicity	A summary value for acute toxicity for bobwhite quail chicks shows an LC_{50}>100,000 mg of grain from Cry1F corn/kg diet (the highest concentration tested). This is equivalent to 10% or 100,000 ppm of the diet being derived from Cry1F corn.	Dimethoate is moderately to very highly toxic to birds. In Japanese quail, a 5-day dietary LC_{50} of 341 ppm is reported. It may be very highly toxic to other birds; reported acute oral LD_{50} values are 41.7 to 63.5 mg/kg in mallards and 20.0 mg/kg in pheasants. Birds are not able to metabolize dimethoate as rapidly as mammals do, which may account for its relatively higher toxicity in these	Chlorpyrifos is moderately to very highly toxic to birds. Its oral LD_{50} is 8.41 mg/kg in pheasants, 112 mg/kg in mallard ducks, 21.0 mg/kg in house sparrows, and 32 mg/kg in chickens. The LD_{50} for a granular product (15G) in bobwhite quail is 108 mg/kg. At 125 ppm, mallards laid significantly fewer eggs. There was no evidence of changes in weight gain, or in the number,	Effects on birds: Permethrin is practically non-toxic to birds. The oral LD_{50} for the permethrin formulation, Pramex, is greater than 9900 mg/kg in mallard ducks, greater than 13,500 mg/kg in pheasants, and greater than 15,500 mg/kg in Japanese quail.

		species.	weight, and quality of eggs produced by hens fed dietary levels of 50 ppm of chlorpyrifos.	
Aquatic Data	There is no evidence for sensitivity of endangered aquatic species to Cry1F delta endotoxin. Low potential for exposure to Cry1F through drifting Cry1F maize pollen or other tissues derived from Cry1F maize and toxicity studies with aquatic invertebrates show very limited hazard for fish or invertebrates exposed to Cry1F. The measured effect level (EC_{50}) for the 48 hr. acute dietary toxicity study with *Daphnia magna* was greater than 100 mg Cry1F pollen/liter. This level is several fold higher than the estimated concentration of 1.25 g Cry1F/liter from pollen drift into fresh water ponds.	Dimethoate is moderately toxic to fish, with reported LC_{50} values of 6.2 mg/L in rainbow trout, and 6.0 mg/L in bluegill sunfish. It is more toxic to aquatic invertebrate species such as stoneflies and scuds.	Chlorpyrifos is very highly toxic to freshwater fish, aquatic invertebrates and estuarine and marine organisms. Cholinesterase inhibition was observed in acute toxicity tests of fish exposed to very low concentrations of this insecticide. Application of concentrations as low as 0.01 pounds of active ingredient per acre may cause fish and aquatic invertebrate deaths. Chlorpyrifos toxicity to fish may be related to water temperature. The 96-hour LC_{50} for chlorpyrifos is 0.009 mg/L in mature rainbow trout, 0.098 mg/L in lake trout, 0.806 mg/L in goldfish, 0.01 mg/L in bluegill, and 0.331 mg/L in fathead minnow]. Chlorpyrifos accumulates in the tissues of aquatic organisms. Studies involving continuous exposure of fish during the embryonic through fry stages have shown bioconcentration values of 58 to 5100. Due to its high acute toxicity and its persistence in sediments, chlorpyrifos may represent a hazard to sea bottom dwellers. Smaller organisms appear to be more sensitive than larger ones .	Effects on aquatic organisms: Aquatic ecosystems are particularly vulnerable to the impact of permethrin. A fragile balance exists between the quality and quantity of insects and other invertebrates that serve as fish food. The 48-hour LC_{50} for rainbow trout is 0.0125 mg/L for 24 hours, and 0.0054 mg/L for 48 hours. As a group, synthetic pyrethroids were toxic to all estuarine species tested. They had a 96-hour LC_{50} of less than or equal to 0.0078 mg/L for these species. The compound has a low to moderate potential to accumulate in these organisms.
Nontarget and beneficial insects	Results indicated that Cry1F delta endotoxin (produced microbially)	Survival of *Microplitis croceipes* (Cresson) adults, parasitoids of the	Aquatic and general agricultural uses of chlorpyrifos pose a	Effects on other organisms: Permethrin is toxic to wildlife. It

	has an acute LC_{50} greater than 320 g Cry 1F/g diet for parasitic Hymenoptera (*Nasonia vitripennis*), and an acute LC_{50} greater than 480 g Cry 1F/g diet for green lacewing (*Chrysoperla carnea*) and lady beetle (*Hippodamia convergens*). These concentrations are several fold higher than the upper bound estimate of 5 g Cry 1F/g pollen derived from line 6275 corn, and indicate low potential for toxicity due to exposure.	cotton pests *H.zea* and *H.virescens*, exposed to residues of insecticides applied at recommended rates to cotton was measured in 1989. In unsprayed cheek treatments, survival was 91.4% after 24 h. The organophosphates profenofos and acephate and the new-generation pyrethroid bifenthrin were highly toxic to *M. croceipes*. All other compounds tested showed some selectivity, including esfenvalerate, cypermethrin, thiodicarb, oxamyl, dicrotophos, dimethoate, and cyhalothrin in order of decreasing survival. The effectiveness of *M. croceipes* as a biocontrol agent of the bollworm and tobacco budworm might be improved through selective use of insecticides to which the parasitoid is tolerant.	serious hazard to wildlife and honeybees.	should not be applied, or allowed to drift, to crops or weeds in which active foraging takes place. The International Organization for Biological Control tested the acute toxicity of permethrin to 13 species of beneficial arthropods and found that permethrin caused 99 percent mortality of 12 of the species, and over 80 percent mortality of the other. Effects were persistent, lasting over 30 days. Sublethal doses also impact beneficial arthropods: permethrin inhibited the emergence of a parasitoid wasp from eggs of the rice moth *Corcyra cephalonica* and disrupted the foraging pattern of another parasitoid wasp as it searched for its aphid prey.
Honey bee toxicity	Petition 00-136-01P by Dow-Mycogen to deregulate Cry1F maize contains details of this analysis in a CBI appendix, and the petition summary indicates an acute dietary toxicity (honey bees) LD_{50}> 640 ng Cry1F/larvae.	Dimethoate is highly toxic to honeybees. The 24-hour topical LD_{50} for dimethoate in bees is 0.12 g per bee	Aquatic and general agricultural uses of chlorpyrifos pose a serious hazard to honeybees.	Permethrin is extremely toxic to bees. Severe losses may be expected if bees are present at treatment time, or within a day thereafter.
Nontarget soil organisms	A 28-day study to determine the chronic effects of microbially-derived CrylF protein on survival and reproduction of Collembola was conducted with three treatment levels of the CrylF test substance (0.63, 3.1, and 12.5 mg/kg of test diet). At	A study of the effects of soil moisture and toxicity of dimethoate was conducted with an enchytraeid worm. Laboratory experiments used dimethoate and small Enchytraeus sp. as the test species. Substrate was natural agricultural field soil cultivated without	Data not found in sources consulted.	Data not found in sources consulted.

	the conclusion of the test, there was less than 10% mortality associated with exposure to either the CrylF protein test substance or the assay control. Reproduction of Collembola was not significantly affected by exposure to the test substance when compared to the assay control. No mortality and no reduction in the number of progeny was observed following exposure to the test materials for 28 days. The results of this study indicate Collembola were not affected by chronic exposure to CrylF at treatment levels exceeding those expected to be found in maize fields based on the calculated worst-case, post-harvest exposure estimates of 0.350 mg Cry1F protein/kg of whole plant material at senescence or 0.063 mg Cry1F protein/kg dry soil. Acute toxicity for earthworm was established by exposure to microbially-produced Cry1F protein in soil. The LC_{50} was > 2.5 mg Cry1F/kg dry soil. This concentration is also considerably higher than the worst-case estimate of Cry1F post-harvest exposure in the soil.	pesticides for several years. Experimental design consisted of three soil moistures (40, 55, and 70% of water holding capacity) and five pesticide concentrations, plus controls. Measured parameters were survival, size of the parent worms and number and size of juveniles produced. Dimethoate was relatively non-toxic to this species. Dimethoate did not decrease survival, but sublethal effects on adult size and number of juveniles were observed. Adverse conditions in dry soil masked these effects; dimethoate appeared to be less toxic in dry soil than in moist soil.		
EPA toxicity class (Class I -highly toxic to Class IV-relatively nontoxic)	Class IV	Dimethoate is a moderately toxic compound in EPA toxicity class II. Labels for products containing dimethoate must bear the Signal Word	Chlorpyrifos is toxicity class II - moderately toxic. Products containing chlorpyrifos bear the Signal Word WARNING or CAUTION, depending	Permethrin is a moderately to practically non-toxic pesticide in EPA toxicity class II or III, depending on the formulation. Formulations are placed

		WARNING. Dimethoate is a General Use Pesticide (GUP).	on the toxicity of the formulation. It is classified as a General Use Pesticide (GUP). The EPA has established a 24-hour reentry interval for crop areas treated with emulsifiable concentrate or wettable powder formulations of chlorpyrifos unless workers wear protective clothing.	in class II due to their potential to cause eye and skin irritation. Products containing permethrin must bear the Signal Word WARNING or CAUTION, depending on the toxicity of the particular formulation. All products for agricultural uses (except livestock and premises uses) are Restricted Use Pesticides (RUPs) because of their possible adverse effects on aquatic organisms.
EDF - Integrated Environmental Rankings[6] - Combined human & ecological scores	not ranked	65 to 100% ranked.on the least to most hazardous scale with 100% being the most hazardous	50 to 75%	0 to 25%
Mammalian toxicity	Toxicology studies conducted to determine the toxicity of Cry1F insect control protein demonstrated that the protein has very low toxicity. In an acute oral toxicity study in the mouse, the estimated acute LD_{50} by gavage was determined to be >5,050 mg of the microbially produced test substance containing 576 mg Cry1F/kg body weight. This dose is 12,190 x greater than the estimated 95th percentile for human dietary exposure to Cry1F protein resulting from consumption of foods derived from Cry1F protected corn. In an in vitro study, Cry1F protein was rapidly and extensively degraded in simulated gastric conditions in the presence of pepsin. This indicates that the potential for adverse	**Acute toxicity:** Dimethoate is moderately toxic by ingestion, inhalation, and dermal absorption. The reported acute oral LD_{50} values for the technical product range from 180 to 330 mg/kg in the rat; although an oral LD_{50} of as low as 28 to 30 mg/kg has been reported. Reported dermal LD_{50} values for dimethoate are 100 to 600 mg/kg in rats, again with a much lower value for an earlier product . Dimethoate is reportedly not irritating to the skin and eyes of lab animals. Severe eye irritation has occurred in workers manufacturing dimethoate, although this may be due to impurities. Via the inhalation route, the reported 4-hour LC_{50} is greater than 2.0 mg/L, indicating slight toxicity. Effects of acute exposure are those typical of	**Acute toxicity:** Chlorpyrifos is moderately toxic to humans. Poisoning may affect the central nervous system, the cardiovascular system, and the respiratory system. It is also a skin and eye irritant. Studies in humans suggest that skin absorption of chlorpyrifos is limited. The oral LD_{50} for chlorpyrifos in rats is 95 to 270mg/kg, 60 mg/kg in mice,1000 mg/kg in rabbits, 32 mg/kg in chickens, 500 to 504 mg/kg in guinea pigs, and 800 mg/kg in sheep. The dermal LD_{50} is greater than 2000 mg/kg in rats, and 1000 to 2000 mg/kg in rabbits. The 4-hour inhalation LC_{50} for chlorpyrifos in rats is greater than 0.2 mg/L. **Chronic toxicity:** Repeated or prolonged exposure to organophosphates may result in the same effects	**Acute toxicity**: Permethrin is moderately to practically non-toxic via the oral route. Via the dermal route, it is slightly toxic, with a reported dermal LD_{50} in rats of over 4000 mg/kg, and in rabbits of greater 2000 mg/kg. Permethrin caused mild irritation of both the intact and abraded skin of rabbits. It also caused conjunctivitis when it was applied to the eyes. The 4-hour inhalation LC_{50} for rats was greater than 23.5 mg/L, indicating practically no inhalation toxicity. **Chronic toxicity**: No adverse effects were observed in dogs fed permethrin at doses of 5 mg/kg/day for 90 days. Rats fed 150 mg/kg/day for 6 months showed a slight increase in liver weights. **Reproductive effects**: The fertility of female

	health effects from chronic exposure is virtually nonexistent. A search of relevant databases indicated that the amino acid sequence of the Cry1F protein exhibits no significant homology to the sequences of known allergens or protein toxins. Thus, Cry1F is highly unlikely to exhibit an allergic response. Collectively, the available data on Cry1F protein along with the safe use history of microbial *Bacillus thuringiensis* products establishes the safety of the plant pesticide *Bacillus thuringiensis* subspecies *aizawai* Cry1F insect control protein and the genetic material necessary for its production in all raw agricultural commodities.	organophosphates. **Chronic toxicity**: There was no cholinesterase inhibition in an adult human who ingested dimethoate for 21 days. No toxic effects and no cholinesterase inhibition were observed in individuals who ingested dimethoate for 4 weeks. Repeated or prolonged exposure to organophosphates may result in the same effects as acute exposure, including the delayed symptoms. **Reproductive effects**: When mice were given 9.5 to 10.5 mg/kg/day dimethoate in their drinking water, there was decreased reproduction, pup survival, and growth rates of surviving pups. **Teratogenic effects**: Dimethoate is teratogenic in cats and rats. It is not likely that teratogenic effects will be seen in humans under normal circumstances. **Mutagenic effects**: Mutagenic effects due to dimethoate exposure were seen in mice. Mutagenic effects are unlikely in humans under normal circumstances. **Carcinogenic effects**: An increase in malignant tumors was reported in rats given oral doses of dimethoate for over a year; but the increases were not dose dependent. Thus the evidence of carcinogenicity, even with high-dose, long-term exposure, is inconclusive. This suggests carcinogenic effects in humans are	as acute exposure including the delayed symptoms. Human volunteers who ingested for 4 weeks 0.1mg/kg/day of chlorpyrifos showed significant plasma cholinesterase inhibition. **Reproductive effects:** Current evidence indicates that chlorpyrifos does not adversely affect reproduction. No effects were seen in 2 studies where animals were tested at doses up to 1.2 mg/kg/day. **Teratogenic effects:** Available evidence suggests that chorpyrifos is not teratogenic. Three studies in pregnant rats or mice indicate that no significant teratogenic effects were seen at doses up to 25 mg/kg/day for 10 days. **Mutagenic effects:** No evidence was found in any of four tests performed that chlorpyrifos is mutagenic. **Carcinogenic effects:** There is no evidence that chlorpyrifos is carcinogenic. There was noincrease in the incidence of tumors when rats were fed 10 mg/kg/day for 104 weeks. **Fate in humans and animals:** Chlorpyrifos is readily absorbed into the bloodstream through the gastro-intestinal tract if it is ingested, through the lungs if it is inhaled, or through the skin if there is dermal exposure. In humans, chlorpyrifos and its principal metabolites are eliminated rapidly. After	rats was affected when they received very high oral doses of 250 mg/kg/day of permethrin during the 6th to 15th day of pregnancy. It is not likely that reproductive effects will be seen in humans under normal circumstances. **Teratogenic effects**: Permethrin is reported to show no teratogenic activity. **Mutagenic effects**: Permethrin is reported to show no mutagenic activity. **Carcinogenic effects**: The evidence regarding the carcinogenicity of permethrin is inconclusive. **Organ toxicity**: Permethrin is suspected of causing liver enlargement and nerve damage. **Fate in humans and animals**: Permethrin is efficiently metabolized by mammalian livers. Breakdown products, or "metabolites," of permethrin are quickly excreted and do not persist significantly in body tissues. Permethrin may persist in fatty tissues, with half-lives of 4 to 5 days in brain and body fat.

		unlikely. **Fate in humans and animals**: Dimethoate is rapidly metabolized by mammals.	a single oral dose, the half-life of chlorpyrifos in the blood appears to be about 1 day.	

1. B.t Cry1F data summary. Petition for Determination of non-regulated status B.t. Cry1F insect -resistant glufosinate-tolerant maize line 1507 (2000) Shanahan, D. and Stauffer, C. Mycogen Seeds, Dow Agrisciences and Pioneer Hi-Bred Intl. Inc. (2000). This petition is assigned APHIS petition number 00-136-01p. The mammalian toxicity profile is derived from the petitioner summary of the pesticide petition to establish an exemption from the requirement of a tolerance for the plant-pesticide *Bacillus thuringiensis* Cry1F protein and the genetic material necessary for its production in plants in or on all food commodities as it appears in the Federal Register: June 15, 2000 (Volume 65, Number 116), pp 37545-37547.

2. Dimethoate Data: Pesticide Information Profiles, EXTOXNET Extension Toxicology Network. Revised June 1996. *http://ace.orst.edu/cgi-bin/mfs/01/pips/dimethoa.htm?8#mfs* ; H. M. Puurtinen, E. A. T. Martikainen (1997) Effect of Soil Moisture on Pesticide Toxicity to an Enchytraeid Worm, Enchytraeus sp., Arch. Environ. Contam. Toxicol. 33:34-41. *http://link.springer-ny.com/link/service/journals/00244/bibs/33n1p34.html*; Survival of Microplitis croceipes (Hymenoptera: Braconidae) in contact with residues of insecticides on cotton. Powell, J.E.; Scott, W.P.(1991) Environmental entomology v. 20 (1): p. 346-348; 1991 Feb.

3. Chlorpyrifos Data: Pesticide Information Profiles, EXTOXNET Extension Toxicology Network. Revised June 1996. http://ace.orst.edu/cgi-bin/mfs/01/pips/chlorpyr.htm.

Chemical Fact Sheet for : Chlorpyrifos, Fact Sheet Number: 37, Date Issued: September 30, 1984 available at *http://pmep.cce.cornell.edu/profiles/insect-mite/cadusafos-cyromazine/chlorpyrifos/index.html*.

4. Permethrin Data: Pesticide Information Profiles, EXTOXNET Extension Toxicology Network. Revised June 1996. *http://ace.orst.edu/cgi-bin/mfs/01/pips/permethr.htm?8#mfs* ; Insecticide Fact Sheet, Coalition for Alternatives to Pesticides/NCAP, P.O.Box 1393, Eugene, Oregon,. J. of Pesticide Reform, Summer, 1998, v. 18, no. 2141. *http://www.safe2use.com/poisons-pesticides/pesticides/permethrin/cox.htm*

5. Lambda-cyhalothrin Data: Pesticide Information Profiles, EXTOXNET Extension Toxicology Network. *http://ace.orst.edu/cgi-bin/mfs/01/pips/lambdacy.htm?6#mfs*.

6. For EDF rankings, Environmental Defense Fund. *http://www.scorecard.org/chemical-profiles/*

Appendix D. Data submitted with the petition in support of non-regulated status for Bt Cry1F corn 6275

Molecular Genetic Characterization
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Southern analysis of the *tet* probe for *cry1F* gene in 6275, Fig. 20, pg. 42.
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Southern analysis of the outside RB probe for *cry1F* gene in 6275, Fig. 24, pg. 46.
Southern analysis of the outside RB probe for *cry1F* gene in 6275, Fig. 25, pg. 47.
Mendelian segregation of B.t. Cry1F maize line for glufosinate tolerance in generations F1, T1F2, BC1F1, BC3F1, BC4F2 and S1 hybrid (tolerant plants were also evaluated for ECB resistance) Table 9, pg. 59. (See also accompanying Fig. 32, pg. 60 with a lineage of the generations in the analysis.)
Cry1F protein levels in tissues from line 6275 hybrids by ELISA , Table 13, pgs. 77-78.
Comparison of Cry1F protein levels in tissues from line 6275 (DAS-06275-8 hybrid) and line 1507 (DAS-01507-1 hybrid) by ELISA , Table 14, pg. 79.
BAR protein levels in tissues from line 6275 hybrids by ELISA , Table 15, pgs. 80-81.
Phenotypic Characterization and Evidence to Support a Lack of Unintended Effects
Efficacy Data, i.e., resistance to lepidopteran (Petition, Section V.E.1).
Agronomic Performance Traits between an line 6275 hybrid, an isogenic Cry1F hybrid and a non-transgenic isogenic hybrid in various field trials across the United States in 2002. 13 Traits evaluated (Petition, Section V.E.2). See also field data reports.
Seed Germination under optimal conditions and under cold stress, Table 27, pg. 102.
Compositional and Nutritional analysis: Whole-plant forage data on proximate analysis (for

protein, fat, fiber, ash and carbohydra). 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 (See Petition 00-136-01P)
Comparison of maize-derived Cry1F protein and microbially-derived Cry1F protein used for bioassays, N-terminal sequence analysis- pg. 52., Glycosylation - CBI Appendix 1, Biological Activity - Table 10, Pg. 54.
Environmental Fate of Cry1F in Soil, CBI Appendix Vol 6., see petition pg. 55.
Colembola - 28 day Chronic exposure study, CBI Appendix 8.,see petition pg. 55.
Honeybee- dietary effects on larvae mortality and development, CBI Appendix 10, see petition pg. 56 amended.
Green Lacewing larvae - Dietary toxicity, CBI Appendix 11., see petition pg. 56 amended.
Parasitic Hymenoptera - Dietary toxicity, CBI Appendix 13., see petition pg. 56 amended.
Ladybird Beetle - Dietary toxicity, CBI Appendix 12., see petition pg. 56 amended.
Daphnia magna - Acute toxicity test, CBI Appendix 9., see petition pg. 56 amended.
Earthworm- Acute toxicity, CBI Appendix 7., see petition pg. 56 amended.
Bobwhite Quail - Dietary toxicity, CBI Appendix 15., see petition pg. 56 amended.
Monarch Butterfly (and other lepidopterans) - Nontarget exposure and risk assessment for dispersal of Cry1F pollen - CBI Appendix 5, see petition pg. 56.
Beneficial arthropod predator - field study conducted in 1999 in Johnston, Iowa, CBI Appendix 16,
Resistance management plan - CBI Appendix 19.

Mice - Acute oral toxicity, CBI Appendix 22.
Allergenicity profile - Comparison of amino acid sequence similarity of Cry1F and PAT proteins to known allergen proteins., CBI Appendix 23.
In vitro digestability of Cry1F - CBI Appendix 24.

Appendix E. Determination of non-regulated status for *Bt* Cry1F corn line 1507.

In response to a petition (designated 03-181-01P) received from Mycogen Seeds c/o Dow AgroSciences LLC and Pioneer Hi-Bred International, Inc., APHIS has determined that genetically-engineered corn line 6275 and progeny derived from it will no longer be considered regulated articles under APHIS regulations at 7 CFR Part 340. Permits or acknowledged notifications that were previously required for environmental release, importation, or interstate movement under those regulations will no longer be required for line 6275 corn and its progeny. Importation of seed of line 6275 corn and its progeny is still, however, subject to the restrictions found in the Foreign Quarantine Notices (regulations at 7 CFR Part 319), just as they apply to other importation of corn seeds. This determination is based on APHIS analysis of field and laboratory data and literature references provided in the petition and other relevant information as described in this environmental assessment that indicate that corn line 6275 and its progeny will not pose a plant pest risk for the following reasons: (1) They exhibit no plant pathogenic properties - although DNA from plant pathogens was used in the development of line 6275 corn, these plants are not infected by these organisms, nor can they incite disease in other plants. (2) They are no more likely to become weeds than insect or herbicide tolerant corn that is currently being cultivated. (3) Introgression from line 6275 corn into wild relatives in the United States and its territories is extremely unlikely and is not likely to increase the weediness potential of any resulting progeny nor adversely effect genetic diversity of related plants any more than would introgression from traditional corn hybrids. (4) They are similar in plant forage composition and in kernel composition and quality characteristics to non-transgenic corn and should have no adverse impact on raw or processed agricultural commodities. (5) They exhibit no potential to have a significant adverse impact on organisms beneficial to agriculture. (6) Compared to current agricultural practices, cultivation of line 6275 corn should not reduce the ability to control insects or weeds in corn or other crops. In addition to our finding of no plant pest risk, there will be no affect on threatened or endangered species under the conditions of the current pesticide registrations granted for Bt field corn.

APHIS also has concluded that there may be new varieties bred from line 6275 corn; however they are unlikely to exhibit new plant pest properties, i.e., properties substantially different from any observed for corn already produced from line 6275 and field tested, or those observed for other corn varieties not considered regulated articles under 7 CFR Part 340.

Michael J. Firko, Ph.D.
Assistant Director, Plant Protection and Quarantine
Animal and Plant Health Inspection Service
U.S. Department of Agriculture
Date: