Notices

Federal Register
Vol. 63, No. 94
Friday, May 15, 1998

DEPARTMENT OF AGRICULTURE

Animal and Plant Health Inspection Service

[Docket No. 97–119–2]

AgrEvo USA Co.; Availability of Determination of Nonregulated Status for Corn Genetically Engineered for Insect Resistance and Glufosinate Herbicide Tolerance

AGENCY: Animal and Plant Health Inspection Service, USDA.

ACTION: Notice.

SUMMARY: We are advising the public of our determination that the AgrEvo USA Company's corn line designated as Transformation Event CBH–351, which has been genetically engineered for insect resistance and glufosinate herbicide tolerance, is no longer considered a regulated article under our regulations governing the introduction of certain genetically engineered organisms. Our determination is based on our evaluation of data submitted by AgrEvo USA Company in its petition for a determination of nonregulated status, an analysis of other scientific data, and our review of comments received from the public in response to a previous notice announcing our receipt of the AgrEvo USA Company's petition. This notice also announces the availability of our written determination document and its associated environmental assessment and finding of no significant impact.

EFFECTIVE DATE: May 8, 1998.

ADDRESSES: The determination, an environmental assessment and finding of no significant impact, the petition, and all written comments received regarding the petition may be inspected at USDA, room 1141, South Building, 14th Street and Independence Avenue SW., Washington, DC, between 8 a.m. and 4:30 p.m., Monday through Friday,

except holidays. Persons wishing to inspect those documents are requested to call before visiting on (202) 690-2817 to facilitate entry into the reading room.

FOR FURTHER INFORMATION CONTACT: Dr. Susan Koehler, Biotechnology and Biological Analysis, PPQ, APHIS, 4700 River Road Unit 147, Riverdale, MD 20737-1236; (301) 734-4886. To obtain a copy of the determination or the environmental assessment and finding of no significant impact, contact Ms. Kay Peterson at (301) 734-4885; e-mail: mkpeterson@aphis.usda.gov.

SUPPLEMENTARY INFORMATION:

Background

On September 22, 1997, the Animal and Plant Health Inspection Service (APHIS) received a petition (APHIS Petition No. 97-265-01p) from AgrEvo USA Company (AgrEvo) of Wilmington, DE, seeking a determination that a corn line designated as Transformation Event CBH-351 (event CBH-351), which has been genetically engineered for insect resistance and glufosinate herbicide tolerance, does not present a plant pest risk and, therefore, is not a regulated article under APHIS' regulations in 7 CFR part 340.

On February 23, 1998, APHIS published a notice in the **Federal Register** (63 FR 8897-8898, Docket No. 97-119-1) announcing that the AgrEvo petition had been received and was available for public review. The notice also discussed the role of APHIS, the Environmental Protection Agency, and the Food and Drug Administration in regulating the subject corn line and food products derived from it. In the notice, APHIS solicited written comments from the public as to whether this corn line posed a plant pest risk. The comments were to have been received by APHIS on or before April 24, 1998. During the designated 60-day comment period, APHIS received 2,271 form letters from farmers expressing support for the subject petition, and a comment letter from a research entomologist at a research unit of the U.S. Department of Agriculture's Agricultural Research Service providing data and information that event CBH-351 corn effectively controls European corn borer (ECB) during all corn developmental stages.

Analysis

Corn event CBH-351 has been genetically engineered to express a Cry9C insect control protein derived from the common soil bacterium *Bacillus thuringiensis* subsp. *tolworthi* (*Bt tolworthi*). The petitioner stated that the Cry9C protein is effective in protecting the subject corn line from damage caused by ECB larvae throughout the growing season. The subject corn line also expresses the *bar* gene derived from the bacterium *Streptomyces hygroscopicus*. The *bar* gene encodes the phosphinothricin acetyltransferase (PAT) enzyme, which, when introduced into the plant cell, confers tolerance to the herbicide glufosinate. The particle bombardment method was used to transfer the added genes into the recipient inbred corn line (PA91 x H99) x H99, and their expression is controlled in part by gene sequences derived from the plant pathogens *Agrobacterium tumefaciens* and cauliflower mosaic virus. While the subject corn line contains the *bla* selectable marker gene, which is normally expressed in bacteria, tests indicate that this gene is not expressed in the plant.

The subject corn line has been considered a regulated article under APHIS' regulations in 7 CFR part 340 because it contains gene sequences derived from plant pathogens. However, evaluation of field data reports from field tests of the corn conducted under APHIS notifications since 1995 indicates that there were no deleterious effects on plants, nontarget organisms, or the environment as a result of the environmental release of corn event CBH-351.

Determination

Based on its analysis of the data submitted by AgrEvo, a review of other scientific data and field tests of the subject corn line, and an analysis of comments from the public on the subject petition, APHIS has determined that corn event CBH-351: (1) Exhibits no plant pathogenic properties; (2) is no more likely to become a weed than corn lines developed by traditional breeding techniques; (3) is unlikely to increase the weediness potential for any other cultivated or wild species with which it can interbreed; (4) will not cause damage to raw or processed agricultural commodities; (5) will not harm threatened or endangered species or other organisms, such as bees, that are beneficial to agriculture; and (6) should not reduce the ability to control insects and weeds in corn or other crops when cultivated. Therefore, APHIS has concluded that the subject corn line and any progeny derived from crosses with other corn varieties will be as safe to grow as corn that is not subject to regulation under 7 CFR part 340.

The effect of this determination is that AgrEvo's corn event CBH-351 is no longer considered a regulated article under APHIS regulations in 7 CFR part 340. Therefore, the requirements pertaining to regulated articles under those regulations no longer apply to the field testing, importation, or interstate movement of the subject corn or its progeny. However, importation of corn event CBH-351 or seeds capable of propagation are still subject to the restrictions found in APHIS' foreign quarantine notices in 7 CFR part 319.

National Environmental Policy Act

An environmental assessment (EA) has been prepared to examine the potential environmental impacts associated with this determination. The EA was prepared in accordance with: (1) The National Environmental Policy Act of 1969, as amended (NEPA)(42 U.S.C. 4321 *et seq.*), (2) regulations of the Council on Environmental Quality for implementing the procedural provisions of NEPA (40 CFR parts 1500-1508), (3) USDA regulations implementing NEPA (7 CFR part 1b), and (4) APHIS' NEPA Implementing Procedures (7 CFR part 372). Based on that EA, APHIS has reached a finding of no significant impact (FONSI) with regard to its determination that AgrEvo's corn event CBH-351 and lines developed from it are no longer regulated articles under its regulations in 7 CFR part 340. Copies of the EA and the FONSI are available upon request from the individual listed under **FOR FURTHER INFORMATION CONTACT.**

Done in Washington, DC, this 11th day of May 1998.

Charles P. Schwalbe,

Acting Administrator, Animal and Plant Health Inspection Service.

[FR Doc. 98-13006 Filed 5-14-98; 8:45 am]

BILLING CODE 3410-34-P

USDA/APHIS Petition 97-265-01p for Determination of Nonregulated Status for *Bt* Cry9C Insect Resistant, Glufosinate Tolerant Corn Transformation Event CBH-351

Environmental Assessment and Finding of No Significant Impact

May 1998

The Animal and Plant Health Inspection Service (APHIS) of the U. S. Department of Agriculture has prepared an environmental assessment before issuing a determination of nonregulated status for a genetically engineered corn transformation event called CBH-351. APHIS received a petition from the AgrEvo USA Company regarding the status of corn transformation event CBH-351 as a regulated article under APHIS regulations at 7 CFR Part 340. APHIS has conducted an extensive review of the petition, supporting documentation, and other relevant scientific information. Based upon the analysis documented in this environmental assessment, APHIS has reached a finding of no significant impact on the environment from its determination that lepidopteran insect resistant and glufosinate herbicide tolerant CBH-351 corn shall no longer be a regulated article.

Joel H Payne for

Rebecca A. Bech
Assistant Director
Scientific Services
Animal and Plant Health Inspection Service
U.S. Department of Agriculture
Date: MAY 8 1998

TABLE OF CONTENTS

I. SUMMARY

The Animal and Plant Health Inspection Service (APHIS), United States Department of Agriculture (USDA), has prepared an Environmental Assessment (EA) in response to a petition (APHIS Number 97-265-01p) from the AgrEvo USA Company (AgrEvo) regarding the regulatory status of genetically engineered (transformed) lepidopteran insect resistant and glufosinate herbicide tolerant corn derived from their transformation event CBH-351 (designated hereafter as CBH-351 corn). This corn is currently a regulated article under USDA regulations at 7 CFR Part 340, and as such, interstate movements, importations, and field tests of CBH-351 corn have been conducted under permits issued or notifications acknowledged by APHIS. AgrEvo petitioned APHIS requesting a determination that CBH-351 corn does not present a plant pest risk, and therefore CBH-351 corn and its progeny derived from crosses with other nonregulated corn should no longer be regulated articles under these APHIS regulations.

The CBH-351 corn has been genetically modified to express a modified *cry9C* gene from *Bacillus thuringiensis* subsp. *tolworthi*. This gene encodes an insecticidal protein that protects the corn plants against the feeding damage of larvae of the lepidopteran insect European corn borer. CBH-351 corn is also genetically modified to express the *bar* gene derived from *Streptomyces hygroscopicus*, which encodes a phosphinothricin-N-acetyltransferase (PAT) enzyme. PAT detoxifies glufosinate and thereby confers tolerance to herbicides based on this active ingredient. An ampicillin resistance gene, *β-lactamase* (*bla*) from *Escherichia coli* has also been introduced in CBH-351 corn, but it is not expressed. The genes were inserted into plasmids which were introduced into the corn genome via the particle bombardment technique.

Field trials of CBH-351 corn have been conducted under the notification procedure (7 CFR Part 340.3). Performance standards for such field trials require that the regulated article and its offspring must not persist in the environment after completion of the test. In accordance with APHIS procedures for implementing the National Environmental Policy Act (NEPA) (7 CFR Part 372), this EA has been prepared prior to issuing a determination of nonregulated status for CBH-351 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.

APHIS has considered the information provided by AgrEvo in its petition as well as other scientific data and information relevant to the potential plant pest risk of CBH-351 corn. A thorough evaluation of the potential for significant impact to the human environment through the unconfined, agricultural use of CBH-351 corn has brought APHIS to a Finding of No Significant Impact (FONSI). The conclusion is based upon the following:

1. CBH-351 corn exhibits no plant pathogenic properties. Although DNA from pathogens were used in their development, these plants are not infected by these organisms nor can these plants incite disease in other plants.

2. CBH-351 corn is no more likely to become a weed than insect or herbicide tolerant corn which has been developed by traditional breeding techniques. 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 CBH-351 corn into wild plants in the United States and its territories is extremely unlikely. Potential introgression from CBH-351 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. CBH-351 corn is substantially equivalent in kernel composition, quality and other characteristics to nontransgenic corn and should have no adverse impact on raw or processed agricultural commodities.

5. CBH-351 corn will not have a significant adverse impact on nontarget organisms, including those beneficial to agriculture; and will not affect threatened or endangered species.

6. Compared to current agricultural practices, cultivation of CBH-351 corn should not reduce the ability to control insects or weeds in corn or other crops.

APHIS believes that CBH-351 corn will be just as safe to grow as corn varieties not subject to regulation under 7 CFR Part 340. APHIS concludes that there will be no significant impact on the human environment if CBH-351 corn and its progeny derived from crosses with other nonregulated corn were no longer considered regulated articles under 7 CFR Part 340.

II. BACKGROUND

Development of CBH-351 corn. AgrEvo has submitted a "Petition for Determination of Non-regulated Status" to the USDA, APHIS (APHIS number 97-265-01p) for genetically engineered corn plants that are resistant against the feeding damage caused by the larvae of the European corn borer (ECB) (*Ostrinia nubilalis* (Hubner)) and are tolerant to glufosinate herbicide. AgrEvo requested a determination from APHIS that corn transformation event CBH-351, and its progeny derived from crosses with other nonregulated corn varieties, no longer be considered regulated articles under 7 CFR Part 340.

ECB damage to corn plants results in stalk lodging, dropped ears, and damaged grain. *B. thuringiensis* 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-endotoxin are used as foliar applied biopesticides. However, they are not routinely effective against ECB because at certain stages the insect primarily feeds inside the plants where the foliar applied biopesticide cannot reach. AgrEvo has modified the corn plant to produce a specific delta-endotoxin protein which is nearly identical to the insecticidally active, trypsin-resistant core of the Cry9C protein that is naturally encoded by the gene *cry9C* derived from B.t. subsp. *tolworthi*. Field testing has demonstrated that CBH-351 corn plants are significantly protected from ECB.

CBH-351 corn has also been transformed with the *bar* gene from the bacterium, *Streptomyces hygroscopicus* that encodes the PAT enzyme. This enzyme is useful as a selectable marker enabling identification of transformed plant cells as well as a source of resistance to the herbicide phosphinothricin (also known as glufosinate, the active ingredient in the herbicides Basta®, Rely®, Finale®, and Liberty®).

The *cry9C* and *bar* genes were fused to noncoding regulatory sequences which enable them to be expressed at high levels, constitutively throughout most of the plant. These regulatory regions were derived from genes from petunia and the plant pathogens cauliflower mosaic virus (CaMV) and *Agrobacterium tumefaciens*, the causative agent of crown gall disease. Two separate plasmids containing these genetic constructs were introduced into the corn genome via particle bombardment. Additional genetic elements present on the transforming plasmids (i.e., the ampicillin resistance gene *β-lactamase* (*bla*) and the origin of replication (*ori*) both from the enteric bacterium, *Escherichia coli*) were also introduced into CBH-351 corn, however these elements are nonfunctional in this organism. Details of the genetic modification are discussed in the Determination (Appendix A, Section IV A.). Because CBH-351 corn is engineered to contain genetic material from plant pathogens, they are considered to be regulated articles under APHIS regulations at 7 CFR Part 340.

CBH-351 corn has undergone field testing in wide variety of locations, in 31 States and territories of the United States since 1995 under notification from APHIS, and in Canada, Belgium, France, Chile and Argentina as well. This field testing was conducted, in part, to confirm that CBH-351 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. AgrEvo has submitted to APHIS field data reports and other relevant data and information upon which to base a determination that CBH-351 corn does not pose a plant pest risk.

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 CBH-351 corn as a regulated article under APHIS regulations. The developer of CBH-351 corn, AgrEvo, submitted a petition requesting that APHIS make a determination that corn transformation event CBH-351, and any progeny derived from crosses of event CBH-351 with other nonregulated corn varieties, no longer be considered regulated articles under 7 CFR Part 340.

APHIS Regulatory Authority. APHIS regulations under 7 CFR Part 340, which were promulgated pursuant to authority granted by the Federal Plant Pest Act, (7 U.S.C. 150aa-150jj) as amended, and the Plant Quarantine Act, (7 U.S.C. 151-164a, 166-167) as amended, 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. CBH-351 corn has been considered a regulated article because some noncoding DNA regulatory sequences were derived from plant pathogens.

Section 340.6 of the regulations, entitled "Petition Process 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.

U.S. Environmental Protection Agency (EPA) and Food and Drug Administration (FDA) Regulatory Authority. CBH-351 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 be registered before distribution or sale, unless exempt by EPA regulation. On August 8, 1997, the EPA announced receipt of an application from Plant Genetics Systems (America), Inc.(PGS), (now a wholly-owned subsidiary of AgrEvo) to register the pesticide product *Bt* Cry9C Corn, a plant-pesticide (EPA File Symbol 70218-R) for protection from the European corn borer and other lepidopteran corn pests containing the active ingredient *B. thuringiensis* subsp. *tolworthi* Cry9C protein and the genetic material necessary for its production in corn (62 *FR* 42784). This active ingredient is not included in any previously registered product. The EPA

has not announced its final decision on this application. 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.

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 September 19, 1997, the EPA announced receipt of the initial filing of a pesticide petition (PP 7F4826), submitted by PGS, proposing an exemption from the requirement of a tolerance for residues of plant-pesticides *B. thuringiensis* subsp. *tolworthi* Cry9C and the genetic material necessary for the production of this protein in or on all raw agricultural commodities (62 *FR* 49224). The EPA has not announced its decision on this petition, but it has however granted a temporary exemption from the requirement of a tolerance for residues of this insecticide in corn for feed use only; as well as in meat, poultry, milk, or eggs resulting from animals fed such feed. This regulation is effective April 10, 1998 (63 *FR* 17687).

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. AgrEvo submitted a summary of their safety assessment to the FDA on March 4, 1998, but their food safety and nutritional consultation with the FDA is not yet complete.

IV. ALTERNATIVES

In the course of preparing the EA for this petition, APHIS considered the following three alternatives: (1) deny the petition, so that CBH-351 corn would continue to be regulated under 7 CFR Part 340, and permits or acknowledgment of notifications from APHIS would still be required for its introduction; (2) approve the petition with geographical limitations; and (3) approve the petition, so that permits or notifications would no longer be required from APHIS under 7 CFR Part 340 for introductions in the United States and its territories of CBH-351 corn or progeny derived from CBH-351 corn and other nonregulated corn. Based on an analysis of the plant pest risk potential and impacts to the human environment from uncontained cultivation of CBH-351 corn, APHIS could find no basis for denying the petition (Alternative 1), or for imposing geographical limitations of the use of CBH-351 corn (Alternative 2).

V. AFFECTED ENVIRONMENT AND POTENTIAL ENVIRONMENTAL IMPACTS

This EA addresses potential environmental impacts from a determination that CBH-351 corn and progeny derived from CBH-351 corn and other nonregulated corn should no longer be considered regulated articles under APHIS regulations at 7 CFR Part 340. This EA considers the genotypic and phenotypic characteristics of CBH-351 corn, and the potential environmental impacts that might be associated with the unconfined cultivation of CBH-351 corn, i.e., cultivation without intentional physical and reproductive confinement from other sexually compatible plants. Additional technical information is included in the determination document appended to this EA, and incorporated by reference. This includes detailed discussions of the biology and taxonomy of corn and its sexually compatible relatives, the genetic components used in the construction of CBH-351 corn, and the analyses that lead APHIS to conclude that CBH-351 corn has no potential to pose a plant pest risk.

Potential impacts based on the relative weediness of CBH-351 corn.

APHIS evaluated whether CBH-351 corn is any more likely to become a weed than the nontransgenic recipient corn line, or other corn currently cultivated, by considering the characteristics of CBH-351 corn, the new traits conferred upon it due to expression of the transgenes, and the characteristics associated with previously deregulated corn engineered to express these transgenes. APHIS also evaluated whether CBH-351 corn was any more likely to transmit weedy characteristic to other cultivated 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 carrying the transformation event CBH-351 exhibits no characteristics that would cause it to be more weedy than the parent corn line. In field tests conducted from 1995 to 1996 in 17 States and territories of the United States, no differences were observed between CBH-351 corn and the nontransgenic counterpart or a non-transgenic standard line for several plant traits or performance measures which might increase the plant's ability 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 CBH-351 corn to become a weed. None of the characteristics of weeds described by Baker involve resistance or susceptibility to insects, and there is no reason to expect that the protection against the target insects provided by this new corn line would release it from any constraint that would result in increased weediness. CBH-351 corn is still susceptible to other non-lepidopteran insect pests and diseases of corn.

Glufosinate-based herbicides are used for post-emergent control of many broadleaf and grassy weeds. In the United States, corn that is grown in rotation with soybeans may volunteer on occasion. Volunteers of CBH-351 corn or offspring of crosses between CBH-351 corn and other corn lines which are not subject to APHIS regulation can 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. APHIS notes in its analysis and determination of nonregulated status for petitions for other glufosinate-tolerant corn lines engineered to express PAT, APHIS determined that these corn lines had no significant potential to become weeds (USDA-APHIS, 1995a, 1995b, and 1997).

APHIS concludes that, with the exception of resistance to certain lepidopteran insects and tolerance to GA herbicides, CBH-351 corn has agronomic traits similar to those of traditionally bred corn, and it does not exhibit traits that would cause increased weediness. Its cultivation should not lead to increased weediness of other cultivated corn.

Potential impacts from gene introgression from CBH-351 corn into its sexually-compatible relatives.

APHIS evaluated the potential for gene introgression to occur from CBH-351 corn to sexually compatible wild relatives and considered whether such introgression would result in increased weediness. Cultivated corn, or maize, *Zea mays* L. subsp. *mays*, is sexually compatible with other members of the genus *Zea*, and to a much lesser degree with members of the genus *Tripsacum* as described in the Determination Section IV.

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

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. Gene introgression from CBH-351 corn into teosinte would require that varieties be developed, and approved for cultivation in locations where these teosintes are located. Since CBH-351 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 varieties of cultivated corn bred for increased resistance to lepidopterans. Teosinte is described to be susceptible to many of the same pests and diseases which attack cultivated corn (Sánchez and Ruiz, 1997, see discussion). It is unlikely that potential introgression of ECB resistance or glufosinate tolerance traits from CBH-351 corn would cause teosinte to become more weedy in the absence of glufosinate herbicide selection.

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 CBH-351 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 it is unlikely that introgression of the *bar* gene would provide a selective advantage to these populations as they would not be routinely subject to herbicide treatments.

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). Our analysis leads us to conclude that there is no reason to expect environmental impacts from CBH-351 corn to be significantly different from those arising from the cultivation of any other variety of insect tolerant or herbicide-tolerant corn.

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

APHIS evaluated the potential for CBH-351 corn plants and their products to have damaging or toxic effects directly or indirectly on nontarget organisms, including those that are recognized as beneficial to agriculture and those that are recognized as threatened or endangered in the United States. APHIS also considered potential impacts on other "nontarget" pests, since such impacts could potentially change agricultural practices. Target pests of the modified Cry9C protein expressed in CBH-351 corn are lepidopteran pests of corn, particularly ECB. Results of field and laboratory studies indicate that no differences were observed for the control of insects other than certain lepidopteran pests.

Based on APHIS analyses of previously deregulated transgenic corn lines (USDA, APHIS, 1995a, 1995b and 1997), the expression of PAT and the presence of the *bla* gene in CBH-351 corn plants is not expected to have deleterious effects or significant impacts on nontarget organisms, including beneficial organisms.

The Cry9C protein expressed in CBH-351 corn is similar to the well known Cry1A class of lepidopteran specific toxins produced by *B.t.* strains. Because 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), they are not expected to adversely effect other invertebrates and all vertebrate organisms, including non-target birds, mammals and humans, because they would not be expected to contain the receptor protein found in the midgut of target insects.

APHIS evaluated the results of several studies reported in the Petition designed to evaluate the sensitivity of representative nontarget organisms to Cry9C as expressed in either whole plant powder or pollen derived from CBH-351 corn plants; or as purified from a Cry-minus *B.t.* bacterial strain engineered to express the protein toxin. Control corn plant powder and pollen test substances lacking insecticidal activity (as assayed against the ECB) were used in these studies to determine whether effects were specific to the CBH-351 transformation event. Data supporting these studies was submitted to the EPA in support of the registration of the plant-pesticide. Test organisms included adult honeybees, a predator ladybird beetle *(H. convergens)*, juveniles of the soil-dwelling invertebrate Collembola (springtails) (*Folsomia candida)*, earthworms, juveniles of the

freshwater invertebrate *Daphnia magna*, Northern bobwhite chicks, and mice. Considering the likely routes of exposure to corn plant tissue or residues of this tissue containing the toxin, and the natural level of exposure expected, APHIS feels that the tests were adequate to evaluate the potential toxic effects on the test organisms which might be expected during cultivation of CBH-351 corn. No effects on these organisms were detected during any of these studies which could be related to the presence of the insecticidal Cry9C protein in CBH-351 corn.

AgrEvo submitted results of a small scale field study conducted in 1996 in Johnston, Iowa that demonstrated that there was no consistent pattern of differences in the number of predators observed on plots planted to either CBH-351 corn or non-transformed genetically similar corn. In addition, the predators observed were just as diverse in both types of plots.

No endangered or threatened lepidopteran insects, as listed in 50 CFR §17.11, feed on corn plants. No other routes for significant exposure to the modified Cry9C toxin exist in the U.S.

Based on this analysis, APHIS concludes that cultivation of CBH-351 corn should not have a significant potential to harm nontarget and beneficial organisms common to agricultural ecosystems, nor should it significantly impact species recognized as threatened or endangered by the U.S. Fish and Wildlife Service.

Potential impacts on biodiversity

Our analysis concludes that CBH-351 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 nonregulated status as requested by AgrEvo in their petition.

Potential impacts on agricultural and cultivation practices

APHIS considered potential impacts associated with the cultivation of ECB-resistant and glufosinate-ammonium tolerant CBH-351 corn on current agricultural practices, in particular, those used to control lepidopteran insect pests and weeds in corn and other crops.

AgrEvo has provided data which indicate that CBH-351 corn expresses modified Cry9C in the relevant tissues across the season at doses which are sufficiently high to provide excellent ECB protection across different environments within the U.S. Corn Belt, and across different genotypes, inbreds, and hybrids. AgrEvo is currently evaluating whether CBH-351 corn can provide field efficacy against

other lepidopteran pests of corn in the United States. CBH-351 corn does not effectively control corn ear worm, and tests have confirmed that it is insensitive to purified Cry9C (personal communication, Sue MacIntosh, Feb. 2, 1998).

CBH-351 corn plants are not likely to eliminate the use of chemical insecticides which are traditionally applied to about 25 to 35% of the total corn acreage planted, since the primary target for most of these applications has been the coleopteran, corn rootworm. CBH-351 corn may positively impact current agricultural practices used for insect control by 1) offering an alternative method for control of ECB (and potentially other Cry9C-susceptible pests of corn); 2) reducing the use of insecticides to control ECB and the resulting potential adverse effects of such insecticides on beneficial insects, farm worker safety, and ground water contamination; and 3) offering a new tool for managing insects that have become resistant to other insecticides currently used or expressed in corn, including other *Bt*-based insecticides. Corn transformation events containing other *Bt* toxins, Cry1Ab and Cry1Ac, have been registered as plant pesticides for the control of ECB (EPA, 1998). Corn varieties containing these toxins have been grown in the U.S. for the past one or two years without any reports of the evolution of resistance to the *Bt* toxins in the target pest. But competition studies of receptor-binding sites suggest that should Cry1Ab- or Cry1Ac- resistant ECB populations eventually evolve, either by the use of *Bt* spray formulations or transgenic plants, they will most likely still be susceptible to CBH-351 corn (Lambert et al., 1996).

Cry9C-resistant populations of previously sensitive insects may eventually develop as a result of feeding on CBH-351 corn plants. APHIS has reviewed a resistance management plan (RMP) that AgrEvo has submitted to the EPA as part of their effort to gain pesticide registration for the insecticidal component as expressed in CBH-351 corn, and have found that it provides numerous viable options for the delay and management of resistance. Even if resistance to Cry9C develops, it is unlikely to significantly impact current agricultural practices used to control ECB or other potentially susceptible pests of corn (even if these pests move to other crops) because 1) Cry9C has never been used commercially for insect control or expressed in transgenic plants, and 2) cross-resistance to other *Bt* toxins currently commercialized in formulations that are most often used in corn and other crops, or which are expressed in plants, is unlikely.

APHIS concludes that cultivation of CBH-351 corn should pose no greater impediments on the control of insects in corn and other crops than the currently practiced methods of ECB control; i.e., the use of ECB-tolerant corn cultivars, including other previously deregulated *Bt* transgenic corn transformation events, and the application of chemical and biologically-based insecticides.

AgrEvo has stated that CBH-351 corn exhibits tolerance to glufosinate ammonium herbicides at concentrations that provide effective weed control and excellent crop safety. CBH-351 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; 2) providing the opportunity to continue to move away from pre-emergent and residually active herbicides; 3) providing a new herbicidal mode of action in corn that allows for improved management of weeds which have developed resistance to herbicides with different modes of action; and 4) decreasing cultivation needs and increasing the amount of no-till acres. Liberty® is currently registered by the EPA for use only on LibertyLink (glufosinate-tolerant) crops - field corn and soybeans. Volunteers of CBH-351 can be easily controlled by selective mechanical or manual weed removal or by the use of herbicides with active ingredients other than glufosinate ammonium.

Potential impacts on raw or processed agricultural commodities.

APHIS analysis of information regarding the disease and insect susceptibility of CBH-351 corn, and data provided on the compositional profiles of the kernels produced on CBH-351 corn plants reveal no differences between CBH-351 and their nontransgenic hybrid counterparts and other standard hybrids that could have a direct or indirect plant pest effect on any raw or processed plant commodity.

Potential environmental impacts outside the United States associated with a determination of nonregulated status as requested by AgrEvo

APHIS has also considered potential environmental impacts outside the United States and its territories associated with a determination of nonregulated status for CBH-351 corn and its progeny as requested by AgrEvo. Several factors contribute to the conclusion that there should be no impacts abroad from cultivation of CBH-351 corn or its progeny.

Any international traffic in corn subject to this determination would be fully subject to national and regional phytosanitary standards promulgated under the International Plant Protection Convention (IPPC). The IPPC has set a standard for the reciprocal acceptance of phytosanitary certification among the nations that have signed or acceded to the Convention (105 countries as of October, 1996). The treaty came into force on April 3, 1952, and establishes standards to facilitate the safe movement of plant materials across international boundaries. Plant biotechnology products are fully subject to national legislation and regulations, or regional standards and guidelines promulgated under the IPPC. The vast majority of IPPC signatories have promulgated, and are now administering, such legislation or guidelines. The IPPC has also led to the creation of Regional Plant Protection Organizations (RPPOs) to facilitate regional harmonization of phytosanitary standards.

Issues that may relate to commercialization of particular agricultural commodities produced through biotechnology are being addressed in international forums.

APHIS has played a role in working toward harmonization of biosafety and biotechnology guidelines and regulations included within the RPPO for our region, the North American Plant Protection Organization (NAPPO), which includes Mexico, Canada, and the United States. NAPPO's Biotechnology Panel advises NAPPO on biotechnology issues as they relate to plant protection.

APHIS participates regularly in biotechnology policy discussions at forums sponsored by the European Union and the Organization for Economic Cooperation and Development. In addition, APHIS periodically holds bilateral or quadrilateral discussions on biotechnology regulatory issues with other countries, most often Canada and Mexico. APHIS also acts as a consultant for the development of biotechnology guidelines and regulations, and has interacted with governments around the world in this manner, including those in regions where corn originated or is cultivated in significant quantities (e.g., Mexico, Brazil, Argentina, and more). We have participated in numerous conferences intended to enhance international cooperation on safety in biotechnology, and sponsored several workshops on safeguards for planned introductions of transgenic crops (crucifers, maize, wheat, potatoes, rice, tomatoes) most of which have included consideration of international biosafety issues. In particular APHIS participated in a recent NAPPO workshop on transgenic maize held in October, 1997 in Mexico in which the potential risks associated with field release of transgenic corn in Mexico were discussed. Mexico possesses many wild *Zea* populations and thus may be concerned with the potential for introgression of genes from domesticated *Zea mays* into these wild populations where such genes may reasonably be expected to have a negative impact. However, conservation measures are already in place to collect germplasm and protect some of these populations *in situ*, and Mexico's regulatory process requires a full evaluation of transgenic plants before they can be introduced into their environment.

In the course of these wide-ranging studies and interactions, APHIS has not identified any impacts on the environment that can not reasonably be mitigated through normal agricultural practices that might be relevant to glufosinate tolerant, lepidopteran insect resistant CBH-351 corn or follow from the unconfined cultivation of such corn in the United States and its territories, or abroad. In addition to the assurance provided by the analysis leading APHIS to a finding of no significant impact for the introduction of this corn, 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 this determination.

VI. CONCLUSION

APHIS has evaluated information from the scientific literature as well as data submitted by AgrEvo that characterized CBH-351 corn. After careful analysis, APHIS has identified no significant impact to the environment from a

determination that CBH-351 corn should no longer be a regulated article under APHIS regulations at 7 CFR Part 340. That finding is supported by the following conclusions: .

1. CBH-351 corn exhibits no plant pathogenic properties. Although DNA from pathogens were used in their development, these plants are not infected by these organisms nor can these plants incite disease in other plants.

2. CBH-351 corn is no more likely to become a weed than insect or herbicide tolerant corn which has been developed by traditional breeding techniques. 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 CBH-351 corn into wild plants in the United States and its territories is extremely unlikely. Potential introgression from CBH-351 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. CBH-351 corn is substantially equivalent in kernel composition, quality and other characteristics to nontransgenic corn and should have no adverse impact on raw or processed agricultural commodities.

5. CBH-351 corn will not have a significant adverse impact on nontarget organisms, including those beneficial to agriculture; and will not affect threatened or endangered species.

6. Compared to current agricultural practices, cultivation of CBH-351 corn should not reduce the ability to control insects or weeds in corn or other crops.

APHIS concludes that CBH-351 corn will be just as safe to grow as corn that are not subject to regulation under 7 CFR Part 340, and that there should be no significant impact on the human environment if CBH-351 corn and its progeny derived from crosses with other nonregulated corn were no longer considered regulated articles under its regulations (7 CFR Part 340).

VII. LITERATURE CITED

Baker, H. G. 1965. Characteristics and modes of origin of weeds, pp. 147-168. *In:* The Genetics of Colonizing Species. Baker, H. G., and Stebbins, G. L. (eds.), Academic Press, New York.

Crockett, L. 1977. Wildly Successful Plants: North American Weeds. University of Hawaii Press, Honolulu, Hawaii. 609 pp.

Doebley, J. 1990a. Molecular evidence for gene flow among *Zea* species. BioScience 40:443-448.

Doebley, J. 1990b. Molecular systematics of *Zea* (Gramineae). Maydica 35(2):143-50.

Galinat, W. C. 1988. The Origin of Corn, pp. 1-31. *In*: Corn and Corn Improvement, Third Edition. Sprague, G. F., Dudley, J. W. (eds.). American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America, Madison, Wisconsin.

Hofmann, C., Luthy, P., Hutter, R., Piska, V. 1988a. Binding of the Delta Endotoxin from *Bacillus thuringiensis* to Brush-Border Membrane Vesicles of the Cabbage Butterfly (*Pieris brassicae*). Eur. J. Biochem. 173:85-91.

Hofmann, C., Vanderbruggen, H., Hofte, H., Van Rie, J., Jansens, S., Van Mellaert, H. 1988b. Specificity of *B. thuringiensis* Delta-Endotoxins is Correlated with the Presence of High Affinity Binding Sites in the Brush-Border Membrane of Target Insect Midguts. Proc. Natl. Acad. Sci. USA 85:7844-7448.

Holm, L., Pancho, J. V., Herbarger, J. P., Plucknett, D. L. 1979. A Geographical Atlas of World Weeds. John Wiley and Sons, New York. 391 pp.

Kato Y., T.A. 1997 Review of introgression between maize and teosinte. *In:* Gene Flow Among Maize Landraces, Improved Maize Varieties, and Teosinte: Implications for Transgenic Maize. pp. 44-53. Serratos, J.A., Willcox, M.C., and Castillo-González, F. (eds.). Mexico, D.F., CIMMYT.

Keeler, K. 1989. Can genetically engineered crops become weeds? Bio/Technology 7:1134-1139.

Lambert, B., Buysse, L., Decock, C., Jansens, S., Piens, C., Saey, B., Seurinck, J., Van Audenhove, K., Van Rie, J., Van Vliet, A., Peferoen, M. 1996. A *Bacillus thuringiensis* insecticidal crystal protein with a high activity against members of the family Noctuidae. Appl. Envir. Microbiol. 62(1):80-86.

Muenscher, W. C. 1980. Weeds. Second Edition. Cornell University Press, New York and London. 586 pp..

Sánchez G., J.J., Ruiz C., J.A. 1997. Teosinte Distribution in Mexico. *In:* Gene Flow Among Maize Landraces, Improved Maize Varieties, and Teosinte: Implications for Transgenic Maize. pp. 18-39. Serratos, J.A., Willcox, M.C., and Castillo-González, F. (eds.). Mexico, D.F., CIMMYT

Van Rie, J. Jansens, S., Hofte, H., Degheele, D., Van Mellaert, H. 1989. Specificity of *Bacillus thruringiensis* δ-Endotoxins, Importance of Specific Receptors on the Brush Border Membrane of the Mid-Gut of Target Insects. Eur. J. Biochem. 186:239-247.

USDA, APHIS. 1995a.USDA/APHIS Petition 94-357-01 for Determination of Nonregulated Status for Glufosinate Resistant Corn Transformation Events T14 and T25: Environmental Assessment and Finding of No Significant Impact. USDA, APHIS, Riverdale, MD.

USDA, APHIS. 1995b. USDA/APHIS Petition 95-145-01 for Determination of Nonregulated Status for Glufosinate Resistant Corn Transformation Line B16: Environmental Assessment and Finding of No Significant Impact. USDA, APHIS, Riverdale, MD.

USDA, APHIS. 1997. USDA/APHIS Petition 96-291-01p for the Determination of Nonregulated Status for Insect-Protected Corn Line DBT418: Environmental Assessment and Finding of No Significant Impact. USDA, APHIS, Riverdale, MD.

U.S. EPA, 1998. The Environmental Protection Agency's White Paper on Bt Plant-pesticide Resistance Management. Biopesticides and Pollution Prevention Division, Office of Pesticide Programs, U.S. EPA, Washington, DC.

Van Rie, J. Jansens, S., Hofte, H., Degheele, D., Van Mellaert, H. 1990. Receptors on the Brush Border Membrane of the Insect MidGut as Determininants of the Specificity of *Bacillus thruringiensis* Delta-Endotoxins. Appl. Environ. Microbiol. 56:1378-1385.

Wilkes, H. G. 1967. Teosinte: the closest relative of maize. Bussey Inst., Harvard Univ., Cambridge, Massachusetts.

Wilkes, H. G. 1977. Hybridization of maize and teosinte in Mexico and Guatemala and the improvement of maize. Econ. Bot. 31:254-293.

Wolfersberger, M.G., Hofmann, C., Luthy, P. 1986. *In* Bacterial Protein Toxins. (eds. Falmagne, P., Alout, J.E., Fehrenbach, F.J., Jeljaszewics, J. And Thelestam, M.) pp. 237-238. Fischer, New York.

VIII. PREPARERS AND REVIEWERS

Operational Support
Scientific Services
Rebecca A. Bech, Assistant Director
James Lackey, Ph.D., Biological Safety Officer
Orrey Young, Ph.D., Arthropods

Biotechnology and Biological Analysis
Subhash Gupta, Ph.D., Biotechnologist (Reviewer)
Susan Koehler, Ph.D., Biotechnologist (Preparer and Petition Coordinator)
David S. Heron, Ph.D., Biotechnologist
Vedpal Malik, Ph.D., Biotechnologist (Reviewer)
Sivramiah Shantharam, Ph.D., Branch Chief
James L. White, Ph.D., Branch Chief (Reviewer)

Safeguarding and Pest Management
Regulatory Coordination and Technical Documentation
Shirley P. Ingebritsen, M.A., Regulatory Analyst (Reviewer)
Michael Schechtman, Ph.D., Senior Microbiologist

IX. AGENCY CONTACT

Kay Peterson, Regulatory Analyst
Scientific Services, Biotechnology Permits
USDA, APHIS
4700 River Road, Unit 147
Riverdale, MD 20737-1237

Telephone: (301) 734-4885
Fax: (301) 734-8669

TABLE OF CONTENTS

Determination of Nonregulated Status for *Bt* Cry9C Insect Resistant and Glufosinate Tolerant Corn Transformation Event CBH-351

Petitioner: AgrEvo USA Company, Wilmington, Delaware
Petition Number: 97-265-01p

Prepared by
United States Department of Agriculture
Animal and Plant Health Inspection Service
Plant Protection and Quarantine
Scientific Services
Riverdale, Maryland

I. SUMMARY

APHIS regulations at 7 CFR Part 340, which were promulgated pursuant to authority granted by the Federal Plant Pest Act (FPPA), (7 U.S.C. 150aa-150jj) as amended, and the Plant Quarantine Act (PQA), (7 U.S.C. 151-164a, 166-167) as amended, regulate the introduction (importation, interstate movement, or release into the environment) of certain genetically engineered organisms and products. An organism is no longer subject to the regulatory requirements of 7 CFR Part 340 when it is demonstrated not to present a plant pest risk. 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.

On September 22, 1997, the Animal and Plant Health Inspection Service (APHIS) received a petition from the AgrEvo USA Company (hereafter referred to as AgrEvo) requesting a determination that corn designated as Transformation Event CBH-351 (hereafter referred to as CBH-351), which has been genetically engineered for insect resistance and tolerance to the herbicide glufosinate, does not pose a plant pest risk and therefore, should no longer be considered a regulated article. AgrEvo submitted supplemental and replacement pages to the petition which were received by APHIS on February 2 and 13, 1998. On February 23, 1998, APHIS announced receipt of the completed petition in the *Federal Register* (63 *FR* 8897-8898) and stated that the petition was available for public review. APHIS invited written comments on this proposed action, to be submitted on or before April 24, 1998. Based on a review of scientific data and literature, APHIS has determined that CBH-351 corn does not present a plant pest risk and is therefore no longer a regulated article under the regulations found at 7 CFR Part 340. As a result of this determination, oversight by APHIS under 7 CFR Part 340 will no longer be required for field testing, importation, or interstate movement of CBH-351 corn or its progeny.

This determination has been made based on an analysis that revealed that CBH-351 corn plants: 1) exhibit no plant pathogenic properties, 2) are no more likely to become a weed than insect resistant and herbicide tolerant corn developed by traditional breeding, 3) are unlikely to increase the weediness potential of any other plant with which they can interbreed, 4) are not likely to cause damage to raw or processed agricultural commodities, 5) are unlikely to harm threatened or endangered species and organisms that are beneficial to agriculture, and 6) are unlikely to reduce the ability to control insect or weed pests in corn and other crops. APHIS has also concluded that there is no reason to believe that new corn varieties derived from CBH-351 corn progeny will exhibit new plant pest properties; i.e., properties substantially different from any observed for the CBH-351 corn already field tested, or those observed for corn in traditional breeding programs.

CBH-351 corn is genetically engineered to express only two additional genes, both of which are derived from different bacteria: (1) a modified version of the *cry9C* gene from *Bacillus thuringiensis* subsp. *tolworthi (B.t. tolworthi)*, that encodes an insecticidal protein; and (2) a *bar* gene from *Streptomyces hygroscopicus* that encodes the enzyme phosphinothricin-N-acetyltransferase (PAT) which confers tolerance to glufosinate herbicides. These genes have accompanying non-coding DNA regulatory sequences that modulate their expression. The DNA regulatory sequences were derived from petunia and the plant pathogens cauliflower mosaic virus (CaMV) and *Agrobacterium tumefaciens*. These genes were introduced into corn on two separate plasmids via particle bombardment. This technique has resulted in the direct incorporation of the plasmids and accompanying genes into the plant genome. Additional genetic elements present on the transforming plasmids, such as the ampicillin resistance gene *β-lactamase* (*bla*) and the origin of replication (*ori*) both from *Escherichia coli*, were also introduced into CBH-351 corn, however these elements are nonfunctional in this organism.

The potential environmental impacts associated with this determination have been examined in accordance with regulations and guidelines implementing the National Environmental Policy Act (NEPA) of 1969 (42 U.S.C. 4321 *et seq.*; 40 CFR 1500-1508; 7 CFR Part 1b; 7 CFR Part 372). An environmental assessment (EA) was prepared and a Finding of No Significant Impact (FONSI) was reached by APHIS for the determination that CBH-351 corn is no longer a regulated article under its regulations at 7 CFR Part 340. This decision does not release CBH-351 corn from regulations administered by the Environmental Protection Agency (EPA) under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136 *et seq.*) and the Federal Food, Drug, and Cosmetic Act (FFDCA) (21 U.S.C. 301 *et seq.*).

The body of this document consists of three parts: (1) background information that provides the legal framework under which APHIS has regulated the field testing, interstate movement, and importation of CBH-351 corn; (2) a summary of comments provided to APHIS on its proposed action during the public comment period; and (3) analysis of the key factors relevant to APHIS' decision that CBH-351 corn does not present a plant pest risk.

II. BACKGROUND

A. APHIS Regulatory Authority

APHIS regulations at 7 CFR 340, which were promulgated pursuant to authority granted by the Federal Plant Pest Act (FPPA), (7 U.S.C. 150aa-150jj) as amended, and the Plant Quarantine Act (PQA), (7 U.S.C. 151-164a, 166-167) as amended, regulate the introduction (importation, interstate movement, or release into the environment) of

certain genetically engineered organisms and products. Under these regulations, a genetically engineered organism is deemed a regulated article if either 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 APHIS has reason to believe that the genetically engineered organism presents a plant pest risk. The FPPA gives the U.S. Department of Agriculture (USDA) the authority to regulate plant pests and other articles to prevent direct or indirect injury, disease, or damage to plants and plant products. In addition, the PQA provides an additional level of protection by enabling USDA to regulate the importation and movement of nursery stock and other plants that may harbor injurious pests.

Before the introduction of a regulated article, a person is required under §340.0 of the regulations to either (1) notify APHIS in accordance with §340.3 or (2) obtain a permit in accordance with §340.4. Introductions under notification (§340.3) must meet specified eligibility criteria and performance standards which impose limitations on the types of genetic modifications that qualify and how the introduction may be conducted, respectively. Under §340.4, a permit is granted for a field trial when APHIS has determined that conducting the field trial, under the conditions specified by the applicant or by APHIS, does not pose a plant pest risk.

An organism is not subject to the regulatory requirements of 7 CFR §340 when it is demonstrated not to present a plant pest risk. 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 the agency determines that the regulated article does not present a risk of introduction or dissemination of a plant pest, the petition will be granted, thereby allowing for unregulated introduction of the article in question. A petition may be granted in whole or in part.

CBH-351 corn has been considered a "regulated article" under §340 of the regulations in part because certain noncoding regulatory sequences were derived from CaMV and *A. tumefaciens*, known plant pests. APHIS believes it prudent to provide assurance before commercialization that organisms such as CBH-351 corn, which are derived at least in part from plant pests, do not pose any potential plant pest risk. Such assurance may aid the entry of new plant varieties into commerce or into breeding and development programs. The decision by APHIS that CBH-351 corn is no longer a regulated article is based in part on evidence provided by AgrEvo concerning the biological properties of CBH-351 corn and their similarity to other varieties of corn grown using standard agricultural practices for commercial sale or private use.

The fact that APHIS regulates genetically engineered organisms having plant pest components does not carry with it the presumption that the presence of part of a plant

pest makes a whole plant a pest or that the plants or genes are pathogenic (McCammon and Medley, 1990). APHIS' approach to plant pest risk is considerably broader than a narrow definition that encompasses only plant pathogens. Other traits, such as increased weediness, and harmful effects on beneficial organisms, such as earthworms and bees, are clearly subsumed within what is meant by direct or indirect plant pest risk. In APHIS' regulations at 7 CFR §340, a "plant pest" is defined as: "Any living stage (including active and dormant forms) of insects, mites, nematodes, slugs, snails, protozoa, or other invertebrate animals, bacteria, fungi, other parasitic plants or reproductive parts thereof; viruses; or any organisms similar to or allied with any of the foregoing; or any infectious agents or substances, which can directly or indirectly injure or cause disease or damage in or to any plants or parts thereof, or any processed, manufactured, or other products of plants."

A determination that such insect-resistant and herbicide-tolerant plants do not present a plant pest risk can be made under this definition, especially when there is evidence that the plants under consideration: 1) exhibit no plant pathogenic properties; 2) are no more likely to become a weed than insect resistant, herbicide tolerant corn developed by traditional breeding; 3) are unlikely to increase the weediness potential of any other cultivated plant; 4) are not likely to cause damage to raw or processed agricultural commodities; 5) are unlikely to harm organisms that are beneficial to agriculture or threatened and endangered species, or to adversely impact the ability to control nontarget insect pests; and 6) are unlikely to reduce the ability to control pests in crops. Evidence has been presented by AgrEvo that bears on these topics. In addition, it should be established that there is no reason to believe that any new corn varieties bred from CBH-351 corn will exhibit plant pest properties substantially different from any observed for corn in traditional breeding programs, or as seen in the development of CBH-351 corn already field tested.

B. EPA and FDA Regulatory Authority

CBH-351 corn is currently subject to regulations and policies administered by the EPA and/or the FDA (described in Section III. of the Environmental Assessment) that require registration of pesticides prior to their distribution and sale, establish tolerances for pesticide residues in raw agricultural products, and establish a process for the safety and nutritional assessment of foods derived from new plant varieties. APHIS' decision on the regulatory status of CBH-351 corn under APHIS' regulations at 7 CFR §340, in no way releases this corn and its progeny from EPA and FDA regulatory oversight.

III. COMMENTS

APHIS has received 2272 comments on AgrEvo's petition during the designated 60-day comment period. One was from a USDA, Agricultural Research Service research

entomologist who neither directly supported or opposed the petition, but requested that APHIS consider data provided which suggests that CBH-351 corn hybrids provide effective control against third and fourth instar ECB during both the vegetative and reproductive stages of corn development. The other comments were form letters from U.S. farmers in support of the petition which recognize CBH-351 corn as a valuable new option in the control of ECB.

IV. ANALYSIS OF THE PROPERTIES OF CBH-351 CORN

A brief discussion of corn biology, taxonomy, and cultivation follows in the next paragraph to help inform the subsequent analysis. This information is expanded in subsequent sections when it is relevant in addressing particular risk assessment issues.

Zea mays L. ssp. *mays* (2n=20), known as maize throughout most of the world, and as corn in the United States, is a large, annual, diploid, monoecious, wind-pollinated grass, that is grown for human consumption, animal feed, silage, vegetable oil, sugar syrups, and other miscellaneous uses. Corn is grown commercially throughout the United States (Jewell, 1989). Corn has been cultivated since the earliest historic times from Peru to central North America. The origin is presumed to be Mexico (Gould, 1968).

Zea is a genus of the family Gramineae (the grass family, alternatively referred to as Poaceae). In addition to cultivated corn, the genus *Zea* also includes several distinct wild taxa, all native to Mexico and Central America, which are collectively referred to as teosintes. All teosintes can be crossed to maize, and they all form fertile hybrids with maize (Doebley, 1990a; Wilkes, 1967; and Jesus Sánchez, Coordinator of Genetic Resources, The Mexican National Institute of Forestry, Agriculture, and Livestock Research, Mexico, personal communication, 1998). The distribution, and the taxonomic and evolutionary background of maize and teosintes have been reviewed (Doebley, 1990a & 1990b; Wilkes, 1997; Sánchez and Ruiz, 1997). Wilkes (1967) presented a classification system for teosinte which provided different geographic populations with racial designations. This classification has been modified by Iltis and Doebley (1980) and Doebley (1990b) to attempt to place the taxa in a sequence which reflects their evolutionary relationships, based on morphological and ecological features and molecular systematics. As presented by Doebley (1990b), *Zea* is divided into two sections, *Zea* and *Luxuriantes* Doebley & Iltis.

Section *Zea*, which includes *Zea mays* ssp. *mays*, also includes its more closely related annual, diploid (2n=20) teosintes: *Z. mays* ssp. *mexicana* (Schrader) Iltis, a large-flowered, mostly weedy teosinte distributed broadly across the central highlands of Mexico; *Z. mays* ssp. *parviglumis* Iltis and Deobley, a small-flowered, mostly wild teosinte of southern and western Mexico; and *Z. mays* ssp. *huehuetenangensis* (Iltis and Doebley) Doebley, a narrowly distributed teosinte of Guatemalan western highlands.

Section *Luxuriantes* contains the other teosintes which are more clearly distinguished from maize by both morphological, biochemical, and cytogenetic characteristics, and which are more narrowly distributed. These include another annual, diploid teosinte, *Z. luxurians* (Durieu and Ascherson) Bird, found in southeastern Guatemala (Doebley, 1990a) and in Oaxaca, Mexico (Sánchez and Ruiz, 1997), and two perennial teosinte species found only in Jalisco, Mexico - the diploid *Z. diploperennis* Iltis, Doebley, and Guzman, and the tetraploid (2n=40) *Z. perennis* (Hitchc.) Reeves and Mangelsdorf.

The closest generic relative to *Zea* is *Tripsacum*, a genus of no fewer than 16 species, most of which are native to Mexico, Central, and South America, and three of which occur in the United States (Gould, 1968; Galinat, Petition Annex 1; Kindiger, Petition Annex 2). Species occurring in the United States include: *T. floridanum* Porter ex Vassy (2n=36) which is native to Southern Florida; *T. dactyloides* including 2n=36 forms which are native to the central and western U.S., and 2n=72 forms which extend along the Eastern seaboard and along the Gulf Coast from Florida to Texas; and *T. lanceolatum* (2n=72) which occurs in the Southwestern U.S. *Tripsacum* differs from corn in many respects, including chromosome number (n=9), in contrast to *Zea* (n=10). All species of *Tripsacum* can cross with *Zea*, but only with difficulty and the resulting hybrids are primarily male and female sterile (Galinat, 1988).

A. The introduced genes, their products, and the added regulatory sequences do not present a plant pest risk in CBH-351 corn.

CBH-351 corn was obtained by transforming the backcrossed hybrid corn line (PA91 x H99) x H99, developed by Plant Genetic Systems, with two pUC19 based plasmids, pRVA9909 and pDE110. These plasmids contain the modified *cry9C* gene and the *bar* gene, respectively, both of which have been engineered for expression in plants. Both plasmids also contain the β-lactamase (*bla*) gene derived from *E. coli*, but this gene is not expressed in CBH-351 corn plant tissues. These genes and the encoded products are described below.

The modified *cry9C* gene (*cry9C.PGS2a*) was derived from the bacterium *Bacillus thuringiensis* subsp. *tolworthi* BTS02618A. The specific *B.t. tolworthi* strain used was isolated from grain dust collected in The Philippines. The gene *cry9C.PGS2a* encodes a truncated protein which corresponds to the insecticidal, 68.7 kDa toxic fragment produced by initial trypsin-digestion of the Cry9C protoxin protein (previously named CryIH). The modifications that have been introduced into the Cry9C protein expressed in CBH-351 corn, as compared to the wild type 129.8 kDa Cry9C protoxin include the following:

1) a C-terminal truncation that removes all the amino acids (aa) following position 666 of the wild type protoxin just after the conserved sequence block 5 shared by certain Lepidoperta-active *Bt* Cry proteins;

2) a N-terminal truncation that removes the first 43 aa at the point corresponding to the N-terminus of the 68.7 kDa fragment of the wild type toxin produced by initial trypsin-digestion, and the subsequent N-terminal addition of the amino acids methionine and alanine;
3) a replacement of arginine by lysine at position 123 in the plant encoded protein which reduces the susceptibility of the protein to trypsin cleavage to a non-toxic 55 kDa fragment.

These modifications do not appear to affect the insecticidal activity of the Cry9C protein against the primary targeted insect, European corn borer (ECB), *Ostrinia nubilalis* (Lambert et al., 1996). The Cry9C toxin is also toxic against other Lepidoptera, including members of the families Pyralidae, Plutellidae, Sphingidae, and Noctuidae.

The *bar* gene was cloned from the soil bacterium *Streptomyces hygroscopicus*. It encodes the enzyme phosphinothricin-N-acetyltransferase (PAT) which inactivates phosphinothricin, the active component in glufosinate-ammonium herbicides. Acetylation of phosphinothricin by PAT results in detoxification of the herbicide. Expression of this enzyme allows for selection of transformed plant cells on selective medium, as well as whole-plant tolerance to glufosinate (DeBlock et al., 1987).

The *bla* gene was present on the plasmid vectors only as a selectable marker for plasmid DNA in the bacterial *E. coli* hosts. When expressed in *E. coli* from a prokaryotic promoter, this gene encodes the enzyme β-lactamase which confers resistance to the antibiotic ampicillin (Yanisch-Perron et al., 1985).

Noncoding DNA regulatory sequences were attached to the modified *cry9C* gene and the *bar* gene in the plasmids to facilitate their expression in plants. These sequences include some which were derived from the plant pathogens cauliflower mosaic virus (CaMV) and *A. tumefaciens*. Specifically, the expression of the modified *cry9C* gene was directed by the 5' constitutive promoter and the 3' region containing the polyadenylation signal both from the 35S transcript of the CaMV, along with the leader sequence of the *cab22L* gene of petunia. The expression of the *bar* gene was also directed by the *35S* CaMV promoter along with the 3' untranslated region from the nopaline synthase (*nos*) gene from *A. tumefaciens* which is involved in transcription termination and polyadenylation. Although most of these regulatory regions were derived from plant pathogens, the regulatory sequences cannot cause plant disease by themselves or with the genes that they are designed to regulate.

The plasmids were introduced into the recipient corn via particle bombardment, a technique that results in direct introduction of DNA into the plant genome. APHIS examined molecular and biochemical analyses which were presented to demonstrate the number of insertion sites, copy number, genetic stability, and expression level of the

transgenes in CBH-351 corn. The conclusions summarized in the Petition (pg. 23) are included below:

Gene	Protein	Is a complete copy present?	Is the protein expressed in the plant?
modified *cry9C*	modified Cry9C	yes - at least 1 copy	yes
bar	PAT	yes - at least 4 copies	yes
bla	ß-lactamase	yes - at least 4-5 copies	no

Southern blot analysis of CBH-351 corn DNA using probes for *cry9C, bar*- 3' nos, *bla*, and the *5'* and *3' 35S* CaMV regulatory sequences in combination with polymerase chain reaction (PCR) analysis demonstrated that at least one copy of the modified *cry9C* gene and four copies of the *bar* gene have been inserted at a single site in the genome (Petition Annex 6). These gene copies (except one of the *bar* genes) are flanked by the *5'* promoter of the *35S* gene, indicating that they should be expressed in most plant tissues. Southern analysis also confirmed the presence of at least 4-5 copies of the *bla* gene, and PCR analysis confirmed that at least one copy of the *bla* gene contains the secretion signal, the active site and the substrate binding site of ß-lactamase. The inserted DNA comprises three fragments which include a single copy of the pDE110 plasmid, a head to tail linked double copy of the pDE110 plasmid and a combined copy of a truncated pDE110 plasmid linked to the pRVA9909 plasmid.

Segregation and linkage analysis indicated that the modified *cry9C* gene and *bar* genes are linked and segregate as a single loci, and the traits are transmitted to progeny in a Mendelian fashion as dominant traits (Petition pg. 29, Table 4.1). Southern analysis data also demonstrated that the transgene locus in Transformation Event CBH 351 corn was stably inherited during crosses of CBH-351 corn into 4 different genetic backgrounds (including corn lines B73, Mo17 and A619) (Petition Annex 6, Fig.10, pg 36), and over 5 generations of backcrossing into corn line H99 (Petition Annex 6, Fig. 11, pg. 37).

Northern analyses and enzyme-linked immunosorbent assays (ELISA) were performed on CBH 351 corn to confirm that the proteins encoded by the modified *cry9C* and *bar* transgenes are expressed as expected, and that the *bla* gene is not expressed. Since the bla gene is not expressed, it is not expected to contribute to the plant's phenotype. When expressed as a percent of total plant protein on a dry weight basis as determined by ELISA, the highest level of modified Cry9C (Petition pg. 33, Table 4.3) was found in the stalk at pollen shed (14%), and it was also expressed at high levels in leaves, roots, and tassels, with lower levels in seeds. PAT was expressed in these same tissues, except the highest levels were in leaves and roots, and lower levels were in stalks, tassels, and seeds. In general, whole plant levels of these proteins were highest at the first two stages tested, vegetative growth and pollen shed, and tended to decline at the

later sampling stages, silage and harvest. ELISA data also demonstrate stable expression of Cry9C and PAT protein in whole plant tissue in hybrids of CBH-351 corn with 4 different genetic backgrounds (Petition pp. 34-35, Tables 4.4 and 4.5).

Expression of the modified Cry9C and PAT are not associated with disease or injury in CBH-351 corn plants or other plants. CBH-351 corn has undergone field testing in a wide variety of locations, States and territories of the United States since 1995 under notification from APHIS, and in Canada, Belgium (see Petition Annex 4), France, Chile and Argentina as well. A summary table of U.S. field trials is provided below.

Year	# States /Territories	# Locations
1995	6- IA, NE, IL, IN, PR, HI	12
1996	11 - IA, IL, IN, MD, NE, KS, MO, MN, WI, PR, HI	25
1997	31 - FL, HI, IL, NE, OH, IN, KS, KY, LA, MS, NC, OK, TN, TX, CA, ND, IA, MD, MN, PR, WI, AZ, HI, MO, NY, SD, CT, GA, PA, CO, AR	335

Agronomic evaluations in 1995 and 1996 (Petition, pg.43, and Annex 8) have shown that CBH-351 corn plants were generally indistinguishable from control corn plants for disease susceptibility and insect susceptibility except for tolerance to European corn borer, where a clear advantage was noted for CBH-351 corn. Susceptibility to stalk-rotting diseases and ear molds (specifically, *Colletotrichum graminicola*, *Fusarium moniliforme*, *Gibberella zeae*, and *Diplodia maydis*), leaf diseases (*Cercospora zeae-maydis*, *Helminthosporium* spp., *Erwinia stewartii*, *Aspergillus flavus*, and *Puccinia sorghi*) and common smut (*Ustillago maydis*) were generally the same in the transgenic and non-transgenic control plants based on visual observations.

The following exceptions were noted in the field data reports. For field tests in Puerto Rico conducted under 95-089-04N (Petition Annex 8 pp. 5-6) some transgenic genotypes exhibited leaf striping of up to 15%. Leaf striping can be caused by a number of factors including certain nutrient deficiencies (e.g. magnesium) and viral diseases that are common to maize in that region (e.g. maize mosaic virus, maize rayado fino virus and maize stripe virus) (Shurtleff, 1980). In some cases (see Petition Annex 8, field data report for 96-094-16N, in Illinois), the incidence of stalk rot was reduced in transgenic lines when compared with non-transgenic controls. Such a result is not unexpected since AgrEvo has presented data which indicate that CBH-351 corn plants have significantly less stalk tunneling damage due to ECB feeding, and therefore would potentially be less susceptible to secondary infection by invading stalk-rot pathogens. Insect activity has long been associated with *Fusarium* infection of maize kernels and stalks, and plant injury caused by such insects as the ECB are often the inital infection sites for *Fusarium* species (Chiang and Wilcoxson, 1961; Christensen and Schneider,

1950; Jarvis et al., 1984). In ECB-resistant hybrids expressing another *B.t.* delta endotoxin, Cry1Ab, in kernels, the incidence and severity of *Fusarium* ear rot and incidence of symptomless kernel infection were reduced compared with near-isogenic hybrids lacking *cry1Ab* genes, particularly when plants were manually infested with ECB (Munkvold et al., 1997).

As further evidence that CBH-351 corn displays no plant pest characteristics, no significant decrease in yield parameters was observed across a wide range of environments and genotypes for either inbreds or hybrid conversions of CBH-351 corn. Yield was roughly the same or improved in the transgenic plants containing CBH-351 germplasm versus their non-transgenic counterparts.

B. CBH-351 corn is not any more weedy than other cultivated corn, nor does it have any significant potential to become a weed or transmit weedy characteristics to other cultivated corn.

APHIS evaluated whether CBH-351 corn is any more likely to become a weed than the nontransgenic recipient corn line, or other corn currently cultivated, by considering the characteristics of CBH-351 corn, the new traits conferred upon it due to expression of the transgenes, and the characteristics associated with previously deregulated corn engineered to express these transgenes. APHIS also evaluated whether CBH-351 corn was any more likely to transmit weedy characteristic to other cultivated corn.

Most definitions of weediness stress the undesirable nature of weeds from the point of view of humans; individual definitions differ in approach and emphasis (Baker, 1965). Baker (1965) defines a plant as a weed if, in any specified geographical area, its populations grow entirely or predominantly in situations markedly disturbed by man (without, of course, being deliberately cultivated). He also described several ideal characteristics of weeds. Although Baker's characteristics have been criticized by some ecologists as nonpredictive, no more broadly accepted suite of characteristics has been defined by ecologists (Williamson, 1994). In our view, there is no formulation that is clearly superior at this time. Keeler (1989) and Tiedje et al. (1989) have adapted and analyzed Baker's list to develop admittedly imperfect guides to the weediness potential of transgenic plants. Both authors emphasize the importance of looking at the parent plant and the nature of the specific genetic changes.

Cultivated corn, including the nontransgenic recipient corn line, lacks most of Baker's "weedy" characteristics (Keeler, 1989). Corn is not listed as a common, serious or principal weed or a weed of current or potential importance in the United States or Canada in most weed literature (Holm et al., 1979; Muenscher, 1955; USDA, 1971; Weed Science Society of America, 1992). APHIS considered data and observations provided in the petition on the agronomic performance, general plant features, and

disease and insect susceptibility of CBH-351 corn evaluated in field tests conducted from 1995 to 1996 in 17 States and territories of the United States (Petition pg. 43, and Annex 8). No differences were observed between CBH-351 corn and the nontransgenic counterpart or a non-transgenic standard line for several plant traits or performance measures which might increase the plant's ability to compete or persist as a weed (e.g., seed germination and seedling emergence, plant vigor, plant height, seed set, and number of volunteers in subsequent seasons).

Expression of the insect control protein, modified Cry9C, in CBH-351 corn will not likely provide a competitive advantage sufficient to cause these to be any more "weedy" than other corn cultivars. AgrEvo states that this insecticidal protein has activity towards ECB, Southwestern corn borer (SWCB) (*Diatraea grandiosella*), black cutworm (BCW) (*Agrotis ipsilon*), and several Armyworm species (*Spodoptera*) (Petition, pg. 47); this is based on bioassays or toxicity assays of neonate larvae fed on artificial diets including the 69.7 kDa toxic fragment of the wild-type Cry9C protein (Lambert et al., 1996), not the lysine mutant as expressed in CBH-351 corn. The Petition states that evaluation of the field efficacy of CBH-351 corn for SWCB, BCW, and Armyworm species is underway, and that for 1998, only ECB will be included on the EPA approved label upon registration of the pesticide. None of the characteristics of weeds described by Baker involve resistance or susceptibility to insects, and there is no reason to expect that the protection against the target insects provided by this new corn line would release it from any constraint that would result in increased weediness. CBH-351 corn is still susceptible to other non-lepidopteran insect pests and diseases of corn.

Expression of the *bar* gene conferring tolerance to L-phosphinothricin-based herbicides in CBH-351 corn will not likely provide a competitive advantage sufficient to cause these plants to be any more "weedy" than other corn cultivars. L-phosphinothricin (L-PPT) is a structural analogue of glutamate, the substrate of the enzyme glutamine synthetase. L-PPT exerts its herbicidal effect by competitively binding and displacing glutamate from the active site of the plant's glutamine synthetase enzyme (Bayer et al., 1972). The PAT enzyme encoded by the *bar* gene catalyzes the conversion of L-PPT to N-acetyl-L-PPT in the presence of acetyl CoA as a co-substrate. This conversion leads to the inactivation of the herbicidal active ingredient because it can no longer bind to and inactivate glutamine synthetase. There is no reason to believe that the PAT enzyme nor the N-acetyl-L-PPT reaction product would cause any changes in the characteristics of CBH-351 corn which would increase its weediness potential relative to other varieties of cultivated corn. APHIS notes that as part of its determination of nonregulated status for petitions for other glufosinate-tolerant corn lines engineered to express PAT (USDA/APHIS petitions 94-357-01p, 95-145-01p, and 96-291-01p), APHIS determined that these corn lines had no significant potential to become a weed (USDA, APHIS 1995a, 1995b, and 1997).

L-PPT is the active ingredient in the commercial herbicide formulations Basta® (Hoechst AG, Germany), Ignite®, Rely®, Liberty® (North America), Finale™ (Hoechst Holland N.V., The Netherlands) and RadicaleX (Imex-Hulst B.V., The Netherlands). The L-PPT in these formulations is synthesized as an ammonium ion salt (common name, glufosinate ammonium [GA]). GA herbicides are used to control both annual and perenial weeds in tree fruits and vines, plantations, and nurseries, and prior to crop emergence in vegetables and field crops. It has recently been registered to control weeds in other crops which have been genetically-engineered for tolerance to GA by expression of the PAT enzyme, including other corn lines which APHIS has deregulated. In the United States, corn occasionally appears as a volunteer in the subsequent growing season, e.g. when corn fields are rotated to soybean production. Selective mechanical or manual weed removal or other herbicides can still be used to control CBH-351 corn should it volunteer as a weed in other crops. CBH-351 corn is still susceptible to other herbicides commonly used to control corn volunteers. For example, in soybeans, herbicides based on sulfonylurea, lipid biosynthesis inhibitors, or Fluazifop/fomesafen combinations can be used to control volunteers of CBH-351 corn (Sue MacIntosh, personal communication to Susan Koehler, April 10, 1998). CBH-351 corn is unlikely, in most cases, to cause a concern if it volunteers in other corn crops.

Gene introgression into other corn cultivars via cross pollination is possible. There is no reason to believe that the genetic constructs introduced during the transformation event would have any effect on the reproductive biology of CBH-351 corn unless the insertion event interrupted a genetic locus critical for the normal reproductive function. AgrEvo reported no differences in the flowering of CBH-351 corn compared to the non-transgenic control plants. If pollen of CBH-351 corn were transferred to any receptive corn stigma within the period of pollen viability, cross-pollination would occur. This potential transfer becomes more unlikely as distance increases from the transgenic plants, and from a practical standpoint becomes increasingly unlikely at a distance much greater than the foundation seed isolation distance of 660 feet. In the U.S., farmers generally purchase hybrid corn seed for planting from a commercial source. Hybrid seed corn, and the inbred lines used to produce it, are grown under appropriate conditions to ensure a high degree of genetic purity. If pollen of CBH-351 corn were to fertilize corn being cultivated for purposes other than for seed for planting, the corn would likely be harvested for products or other uses and would not likely be used as seed.

If CBH-351 corn crosses with corn lines expressing resistance to herbicides with a different mode of action (e.g. glyphosate-resistant corn lines deregulated by APHIS determinations for petitions 97-099-01p and 96-317-01p, and imidizolinone or sulfonylurea-tolerant corn varieties), corn volunteers with multiple herbicide resistance might emerge, however the frequency of such occurrences will be reduced by differences in flowering times of different cultivars, distance between fields, and competition from the pollen load within a given field. Various agronomic practices,

including tillage, appropriate variety selections and crop rotation, and rotation of herbicides with different modes of action can be used as appropriate to avoid and or manage volunteer corn resistant to one or a few herbicides, including CBH-351 corn.

Based on this analysis, APHIS concludes that, with the exception of resistance to certain lepidopteran insects and tolerance to GA herbicides, CBH-351 corn has agronomic traits similar to those of traditionally bred corn, and it does not exhibit traits that would cause increased weediness. Its cultivation should not lead to increased weediness of other cultivated corn.

C. Gene introgression from CBH-351 corn into its sexually compatible relatives should not increase the weediness potential of resulting progeny any more than gene introgression from other traditional corn hybrids.

APHIS evaluated the potential for gene introgression to occur from CBH-351 corn to sexually compatible wild relatives and then considered whether such introgression would result in increased weediness.

The reproductive biology of corn and the distribution and sexual compatibility of its closest relatives was discussed previously (Determination Section IV, pg. 5). 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 (populations reported in Honduras are now extinct) (Jesus Sánchez, personal communication, 1998); however, teosinte is known to have survived as an escape from cultivation in Florida and Texas, and a fairly rare, sparsely dispersed population of such has been reported in Florida (Petition, Annex 1 and Annex 2). In Mexico, Guatemala, and Nicaragua, most teosinte populations exist as opportunistic plants 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 in their excrement. In some cases, the same teosinte races or species which are considered to be weeds are also used by some farmers for maize breeding and improvement, for example this is true for the Balsas race *Z. mays* ssp. *parviglumis* in Jalisco Mexico (Sánchez and Ruiz, 1997, and references therein).

Teosinte, both diploid and tetraploid members, can be crossed with cultivated corn to produce fertile F_1 hybrids (Doebley, 1990; 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, some more frequently than others. For example for 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). The perennial species, *Z. perennis* and *Z. diploperennis* have been used as sources of genes

to increase heterosis and variability in maize (Magoja and Pischeda, 1986), and fertile hybrids can be found in natural populations of both species; however very few are found in *Z. perennis* (J. Sánchez, personal communication, 1998). Research comparing frequencies of shared traits (such as different chromosome knobs, isoenzymes, and chloroplast DNA) between sympatric populations of maize and teosinte suggests introgression has occurred in the past (KatoY., 1997 and references therein). In particular, isozyme data have supported (but not conclusively demonstrated) that introgression has occurred from maize to *Z. mays* ssp. *luxurians* and *Z. mays* ssp. *diploperennis* and from annual Mexican plateau teosinte (*Z. mays* ssp. *mexicana*) to maize. Besides introgression, other possible explanations for the occurrence of shared traits include retention of ancestral traits and convergent or parallel evolution.

Introgression from teosinte may in some cases be used to increase the hybrid productivity of corn and serve as a source of pest resistance genes (particularly viral and fungal resistance) (Galinat, Petition Annex 1). Nonetheless, in the wild, introgressive hybridization from corn 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 between maize and teosinte 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. Gene introgression from CBH-351 corn into teosinte would require that varieties be developed, and approved for cultivation in locations where these teosintes are located. Since CBH-351 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 varieties of cultivated corn bred for increased resistance to lepidopterans. Teosinte is described to be susceptible to many of the same pests (including coleopteran, lepidopteran, and homopteran insects) and diseases which attack cultivated corn (Sánchez and Ruiz, 1997, see discussion). It is unlikely that resistance to ECB or tolerance to glufosinate ammonium herbicides due to potential gene introgression from Transformation Event CBH-351 would cause teosinte to become more weedy in the absence of glufosinate ammonium herbicide selection.

The second closest relative of corn is the genus *Tripsacum*. Most of the 13 to 16 different *Tripsacum* species recognized are native to Mexico, Central and South America, but three occur in the U.S.. *Tripsacum dactyloides* (Eastern gamagrass) is currently also being cultivated in the midwest U.S. as a new forage or laylage crop (Kindiger, Petition Annex 2; Galinat, Petition Annex 1). Even though many of these *Tripsacum* species occur in areas where corn might be cultivated, gene introgression from CBH-351 corn under natural conditions is highly unlikely, if not impossible. Hybrids of *Tripsacum* species with *Zea* are difficult to obtain outside of a laboratory and are often sterile or have greatly reduced fertility. Only 10-20% of all maize-Tripsacum

hybrids will set seed when backcrossed by maize, and none are able to withstand even the mildest winters. The only known case of a naturally occurring "Zea" - Tripsacum hybrid is a species native to Guatemala known as *Tripsacum andersoni*. It is 100% male and nearly 99% female sterile (Kindiger, Petition Annex 2). Furthermore, none of the sexually compatible relatives of corn in the U.S. are considered to be serious, principal, or common weeds in the U.S. (Holm et al., 1979); therefore it is unlikely that introgression of the *bar* gene would have any impact on their populations as they would not be routinely subject to herbicide treatments.

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). Our analysis leads us to conclude that there is no reason to expect environmental impacts from CBH-351 corn to be significantly different from those arising from the cultivation of any other variety of insect-tolerant or herbicide-tolerant corn.

D. Use of CBH-351 corn should have no more adverse impacts on raw or processed agricultural commodities than the parent corn.

During field testing, CBH-351 corn exhibited the typical agronomic characteristics of the recipient plant, with the exception of the desired phenotypes conferred by the *bar* and modified *cry9C* genes. No unexpected differences were consistently observed in disease and pest susceptibilities during field observations. APHIS also examined data provided on the compositional profiles of the kernels produced on CBH-351 corn plants compared to their nontransgenic hybrid counterparts and other standard hybrids. Proximate analysis results indicated that hybrids with event CBH-351 corn exhibited minor but significant differences for crude protein and crude fiber, although these values were either similar to the other standard hybrids in the test or within the range of that calculated from USDA-HNIS data. No differences were noted for percentages of moisture, fat/oil, or ash (Petition, Table 5.5). Based on this analysis, characteristics of CBH-351 corn reveal no differences that could have an indirect plant pest effect on any raw or processed plant commodity.

There has been some concern that the *bla* gene may be transmitted horizontally (nonsexually, for example through gene exchange in the food chain) to bacteria that cause diseases. But the general sense among scientists was empathically endorsed by a conference of fourteen scientists and international food policy experts (including representatives of the World Health Organization and the Food and Agricultural Organization) which concurred that the use of the ampicillin marker gene in corn constitutes an insignificant to near zero risk of causing ampicillin resistance complications in either animals or humans (Foundation for Nutritional Advancement, 1996).

APHIS notes that the food and feed safety of the insecticidal component expressed in CBH-351 corn and the use of glufosinate ammonium herbicides on CBH-351 corn are subject to regulations administered by the U.S. EPA, and food and feed use are subject to regulatory oversight by the FDA (see the Environmental Assessment).

E. CBH-351 corn exhibits no significant potential to harm organisms beneficial to the agricultural ecosystem or threatened or endangered species.

Consistent with its statutory authority and requirements under NEPA, APHIS evaluated the potential for CBH-351 corn plants and plant products to have damaging or toxic effects directly or indirectly on nontarget organisms. This includes those that are recognized as beneficial to agriculture and those that are recognized as threatened or endangered in the United States. APHIS also considered potential impacts on other "nontarget" pests, since such impacts could have an impact on the potential for changes in agricultural practices. Target pests of the engineered modified Cry9C protein expressed in CBH-351 corn lines are lepidopteran pests of corn, particularly the European corn borer. Field data reports, and results of field studies and laboratory studies submitted by AgrEvo (Petition Appendix 8) indicate that no differences were observed for the control of insects other than certain lepidopteran pests.

There is no reason to believe that expression of PAT in CBH-351 corn plants would have deleterious effects or significant impacts on nontarget organisms, including beneficial organisms. APHIS analysis of data and literature supporting the nonregulated status of other corn lines which also express PAT, did not reveal any nontarget effects associated with its expression in corn tissue (USDA, APHIS, 1995a, 1995b and 1997). There is also no reason to believe that the *bla* gene in CBH-351 corn would have any impact on non-target organisms since this gene does not encode an infectious agent and the gene is not expressed in the plant.

Data provided with the petition and the scientific literature and our review of field data and toxicity data submitted to the EPA in support of the registration of the plant-pesticide indicate that cultivation of CBH-351 corn expressing the modified Cry9C insecticidal protein should not have a significant potential to harm nontarget organisms common to agricultural ecosystems, nor should it have a significant impact on species recognized as threatened or endangered by the U.S. Fish and Wildlife Service. The rationale for this assessment follows.

1) Potential impact on beneficial and other nontarget organisms.

While *Bt* -based products have been used for insect control for over 30 years, only four different strains have been commercialized, and these do not include the *Bt tolworthi* strain from which the *cry9C* gene was derived. The Cry9C protein encoded by this

strain is similar to the well known class of Cry1A proteins which display activity towards specific lepidopteran larvae through a series of steps involving crystal and protoxin processing, high affinity binding of the toxin to specific receptors in the brush border membranes of larvae midgut, membrane insertion, and pore formation. The activity spectrum of Cry9C is described as being most similar to that of Cry1Cax (Lambert, et al., 1996). Because these Cry proteins bind 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), they are not expected to adversely effect other invertebrates and all vertebrate organisms, including non-target birds, mammals and humans, because they would not be expected to contain the receptor protein found in the midgut of target insects.

APHIS evaluated the results of several studies reported in Chapter 6 C. of the Petition designed to evaluate the sensitivity of representative nontarget organisms to Cry9C as expressed in different test substrates: modified Cry9C protein contained in either whole plant powder or pollen derived from CBH-351 corn plants that were hemizygous for the Cry9C insertion; or Cry9C purified from a Cry-minus *B.t.* bacterial strain engineered to express the protein toxin. The bacterially-produced Cry9C protein is identical to the transgenic plant expressed protein save for the first two amino acids at the N-terminus. Both proteins have the single amino acid change from Arg to Lys at amino acid number 123 of the plant-encoded protein (or the aa # 165 of the full-length protoxin). The microbially-produced truncated toxin protein with this amino acid change was used in the Mouse Oral Toxicity Study and Collembola study discussed below. Data supporting these studies was submitted to the EPA in support of the registration of the plant-pesticide (See Petition Annex 9 for a list of the studies). Pollen test substances contained only 0.24 µg modified Cry9C per gram. Modified Cry9C in lyophilized whole plant powder from 10 week old CBH-351 corn plants was present at approximately 1500 fold higher concentrations, i.e., 2.3% of the total extractable protein or 359 µg/g of powder, and it was highly insecticidal with a LC50 of 31.3 ng/cm^2 in a quantitative feeding bioassay with ECB larvae (MRID # 442581-05). Bacterially produced Cry9C had a similar LC50. Control corn plant powder and pollen test substances used in these studies were derived from the same population of corn plants segregating for the absence of the transformation event, and these lacked insecticidal activity as determined by the same ECB bioassay. The results of these studies (and other relevant studies) on the representative nontarget organisms (including predators, pollinators, soil-dwelling insects and earthworms, aquatic insects, and avian and mammalian wildlife) is discussed as it relates to the cultivation of CBH-351 corn.

Beneficial Insects - Pollinators and Predators

Honeybee (*Apis mellifera*) - Adults from one to five days old were exposed to control or transgenic corn pollen at a concentration of 24,000 µg/ml in an artificial diet and observed over 8 days for mortality and signs of toxicity or abnormal behavior. This is equivalent to an exposure rate of 5.8 ng of modified Cry9C/ml in the transgenic pollen-

containing diet. No effects were detected which were specifically related to the presence of transgenic pollen (MRID # 443843-02). Adult honeybees primarily feed on nectar, they do not forage on corn plants; therefore CBH-351 corn or any other corn is not expected to be a food source for them. Pollen collected on the hairy bodies of solitary and social bees, does however provide an important part of the diet for their larvae, and corn pollen picked up on the wind could be included as part of that diet, although the concentration would be expected to be low compared to pollen collected from plants on which bees had been specifically foraging. The average weight of the pollen load from a given foraging trip has been reported to be 8 to 29 mg per load for different crops (Free, 1970). Therefore the level of pollen tested is more than adequate to evaluate the effects on adult bees of incidental exposure to transgenic pollen which might be expected during cultivation.

Ladybird Beetle (*Hippodamia convergens*) - Adults were exposed to control or transgenic corn pollen at a concentration of 1,500 µg/ml in an artificial diet and observed over 21 days for mortality and signs of toxicity or abnormal behavior. This is equivalent to an exposure rate of 0.36 ng of modified Cry9C/ml in the transgenic pollen-containing diet. No effects were detected which were specifically related to the presence of transgenic pollen (MRID # 443843-02). Adult ladybird beetles are important predators of a great variety of agricultural pests, particularly aphids. Coccinellidae species, particularly *Coleomegilla maculata*, were the most frequently observed predators in a field study (discussed below) designed to observe the effects of CBH-351 corn on insect predators. Other Coccinellidae species, including *H. convergens*, were also observed in that study (MRID # 442581-15). *H. convergens*, is primarily a predator on aphids and lepidopteran eggs, but since Coccinellidae predators are also known to eat pollen, particularly *C. maculata* (which is rather omnivorous) (Hagen, 1987, and references therein); and since corn pollen may be present on prey eaten by Coccinellidae predator species, the choice of pollen as a test substrate and the level tested should be adequate to evaluate the effects on adult ladybird beetles of incidental or direct exposure to transgenic pollen which might be expected during cultivation.

A field study conducted in 1996 by the Iowa State University on a site in Johnston, Iowa also demonstrated that there was no consistent pattern of differences in the number of predators observed on plots planted to corn containing the Transformation Event CBH-351 (50% of the population) versus plots planted to non-transformed genetically similar corn (MRID # 442581-15). In this study, three replicate plots of 10 rows of 40 foot length, half of which received a synthetic non-selective insecticide treatment (Pounce) and half of which were untreated, were observed for predators from early July until September 3 by a biweekly 5-minute observation period in which all predator life stages were counted and by a weekly analysis of sticky traps. While the number of captured predators was significantly different (higher) in the nontransgenic control plots for three of the seven sticky trap harvest dates, this pattern was not

consistent, in that there were no significant differences between the nontransgenic control plots and the CBH-351 corn plots in the number of predators harvested from sticky traps from the other four harvest dates and there were no significant differences detected between these two treatments in any of the five biweekly observations. No significant differences were observed between the sprayed and unsprayed plots for any of the seven sticky trap observation dates and for four of the five 5-minute observation periods; this result could be complicated by the small plot size. The types of predators observed were just as diverse in plots containing CBH-351 corn as they were in plots containing nontransgenic controls. The most abundant predators observed over both methods of observation were the Coccinellidae species (particularly *Coleomegilla maculata*, but also *Hippodamia convergens*, *H. tredecimpunctata*, and *Coccinella septempunctata*). Other predators observed include a member of the Minute Pirate Bug family (Anthocoridae) *Orius insidious* (an important predator on the eggs and larvae of the corn earworm); *Nabis* spp., members of the Damsel Bug family (Nadiae); *Chrysoperla* spp. and *Chrysopa* spp., members of the Common Lacewing family (Crysopidae); members of the Syrphid Fly family (Syrphidae); and Arachnida (spiders).

Soil Dwelling Invertebrates

Transformation Event CBH-351 corn may release pesticidal proteins into the soil when the plant tissue is left on the soil. An environmental fate study (MRID# 441617-01) indicates that transgenic plant powder biologically degrades rapidly when mixed with soil. It is estimated that the modified Cry9C in this tissue reaches 50% of its original insecticidal activity following 4.5 days. This rapid biodegradation is consistent with previous results with Cry1Ab protein. Given the expected rapid biodegradation rate, and the lower level of modified Cry9C present in CBH-351 corn following harvest, prolonged exposure of soil-dwelling invertebrates to highly active concentrations of modified Cry9C in decaying plant material is unexpected. Even so, toxicity tests with Collembola and earthworms confirm that no effects are expected at high exposure rates. Collembola (springtails) (*Folsomia candida*) are soil-dwelling invertebrates that feed on decaying plant material. Juveniles were exposed for 28 days to 50%, 5%, and 0.5% by weight of transgenic plant powder or bacterially-produced modified Cry9C in a food source, and no statistically significant effects were consistently observed that were related to the modified Cry9C treatment over the exposure period (MRID# 44258110). Earthworm mortality, body weight, and behavior were also unaffected by a 14 day exposure to transgenic plant powder incorporated at the high rate of 4,975 mg per kg dry soil (equivalent to 1.84 mg modified Cry9C/kg soil) (MRID# 44258113).

Aquatic Invertebrates

Since transgenic corn plant material is expected to biodegrade rapidly, the primary exposure route of aquatic invertebrates would be through pollen drift onto fresh water ponds, lakes and streams adjacent to fields under cultivation with CBH-351 corn. Juveniles of the freshwater invertebrate *Daphnia magna*, when exposed to transgenic corn pollen at the high rate of 150 µg/ml of well water for 48 hours, were uneffected in

terms of mortalities, immobility, or signs of toxicity or abnormal behavior as compared to Daphnids exposed to nontransgenic control pollen.

Avian and Mammalian Wildlife

Birds could be exposed to the modified Cry9C protein indirectly by feeding on pests of Transformation Event CBH-351 corn or directly by eating the kernels. A five day dietary toxicity study with Northern Bobwhite hatchlings demonstrated that the no observed effect level for transgenic plant powder of CBH-351 corn was considered to be greater than 20% w/w (equivalent to 58 µg modified Cry9C/g of diet). Transgenic plant powder was chosen as a substrate instead of kernels because of the higher modified Cry9C concentration.

Mice and other rodents might also feed on kernels of CBH-351 corn. The acute oral LD50 of transgenic plant-equivalent Cry9C protein as prepared from Cry minus -*Bt* engineered to express Cry9C (which is at least 85% pure) was estimated to be greater than 6500 mg/kg of body weight (MRID# 442581-07). This would correspond to a dose of approximately 171 mg for an average 31 g mouse. Considering the low concentration of modified Cry9C determined for kernels of CBH-351 corn (0.0186 mg/g dry weight), a mouse would have to consume massive amounts (more than 9 kg) to receive this dose of modified Cry9C. The EPA has recently established a temporary exemption from the requirement of a tolerance for residues of the insecticide, *B. thuringiensis* subspecies *tolworthi* Cry9C protein and the genetic material necessary for its production in corn for feed use only; as well as in meat, poultry, milk, or eggs resulting from animals fed such feed. This regulation became effective April 10, 1998 [Federal Register: April 10, 1998 (Volume 63, Number 69) Page 17687-17690].

2). Potential impact on threatened and endangered arthropods.

No endangered or threatened lepidopteran insects, as listed in 50 CFR §17.11 feed on corn plants.

Therefore, APHIS concludes that Transformation Event CBH-351 corn will not have a significant adverse impact on organisms beneficial to plants or agriculture or nontarget organisms, and it will not affect threatened or endangered species.

F. Cultivation of CBH-351 corn should not reduce the ability to control insects and weeds in corn and other crops.

APHIS considered potential impacts associated with the cultivation of ECB-resistant and glufosinate-ammonium tolerant CBH-351 corn on current agricultural practices, in particular those used to control insects and weeds.

In the U.S. Corn Belt, the ECB produces 1-3 generations per year. Larvae feeding, depending on the larvae stage and generation, causes damage to leaf whorls and the sheath collar, tassels and anthers, stalks, shanks, and cobs, and results in reduced plant growth, stalk lodging, ear droppage, and increased risk of secondary infections by bacteria and fungi, all of which reduce grain yield annually by 2 - 8%. ECB and other corn borers (such as SWCB) are difficult to control with non-systemic chemical insecticides because they are vulnerable for only a short time before they bore into and are protected by the plant. AgrEvo has provided data which indicate that CBH-351 corn expresses modified Cry9C in the relevant tissues across the season which are sufficiently above the LC50 determined for neonate ECB larvae to be considered a high dose, at least for those populations tested (Petition Annex 4). Data provided from comments to the petition indicate that ECB is effectively protected from third and fourth instar feeding as well. Data from field trials conducted in 1995 and 1996 demonstrated that CBH-351 provided excellent ECB protection across different environments within the U.S. Corn Belt, across different genotypes and for both inbreds and hybrids (Petition, pp 38-42). Therefore, AgrEvo claims that because the insecticidal protein is expressed at sufficiently high levels at the proper time and in the proper tissues in CBH-351 corn, one can avoid the inherent problems associated with external insecticide applications used to control ECB larvae.

AgrEvo is currently generating data to determine whether CBH-351 corn can provide field efficacy against other lepidopteran pests of corn such as black cutworm, which is a polyphagous pest distributed across most of the United States, and Southwestern cornborer, which is a cold-sensitive corn pest distributed primarily in the south central United States. Toxicity tests with Cry9C have shown that the common cutworm (*A. segetum*) is sensitive, and preliminary experiments indicate that black cutworm has some sensitivity (Lambert et al., 1996). CBH-351 corn does not effectively control corn ear worm (*Heliocoverpa zea*), and toxicity tests have confirmed that it is insensitive to the purified Cry9C protein (personal communication from Sue MacIntosh, Feb. 2, 1998).

CBH-351 corn plants are not likely to eliminate the use of chemical insecticides which are traditionally applied to about 25 to 35% of the total corn acreage planted, since the primary target for most of these applications has been the coleopteran, corn rootworm. But perhaps they may encourage more selective use of insecticides against these pests. CBH-351 corn may positively impact current agricultural practices used for insect control by 1) offering an alternative method for control of ECB (and potentially other Cry9C-susceptible pests of corn); 2) reducing the use of insecticides to control ECB and the resulting potential adverse effects of such insecticides on beneficial insects, farm worker safety, and ground water contamination; and 3) offering a new tool for managing insects that have become resistant to other insecticides currently used or expressed in corn, including other *Bt*-based insecticides. Three Cry1Ab corn transformation events and one Cry1Ac corn tranformation event have been registered as plant pesticides for the control of ECB (EPA, 1998). Competition studies of receptor-

binding sites have shown that Cry9C recognizes a receptor in ECB brush border membrane vesicles that is different from that recognized by Cry1Ab and Cry 1Ac; and a Cry1Ab5-resistant colony of diamondback moth (*Plutella xylostella)* is still sensitive to Cry9C (Lambert et al., 1996). Therefore, should Cry1Ab- or Cry1Ac- resistant ECB populations evolve, either by the use of *Bt* spray formulations or transgenic plants, they will most likely still be susceptible to CBH-351 corn.

Cry9C-resistant populations of previously sensitive insects may eventually develop as a result of feeding on CBH-351 corn plants. However, AgrEvo is aware of the possibility of insect resistance to this transgenic corn, and they have submitted a resistance management plan (RMP) to the U.S. EPA as part of their effort to gain pesticide registration for the insecticidal component as expressed in CBH-351 corn (application EPA File Symbol 70218-R), and they have stated that they will implement a resistance management strategy as the acreage planted to Transformation Event CBH-351corn increases (MRID 442581-16). APHIS has reviewed this RMP and have found that it provides numerous viable options for the delay and management of resistance. An anaylsis of resistance management strategies for other ECB-resistant *Bt* plants and conditions placed on the conditional registrations of these plant pesticides has been provided by the U.S. EPA (U.S. EPA, 1998). The U.S. EPA is likely to impose additional conditions to address any inadequacies of the RMP as part of the registration conditions similar to those imposed upon other companies registering *Bt* corns as plant-pesticides (Sharlene Matten, Pesticide Resistance Management Workgroup, U.S. EPA, personal communication to Susan Koehler, 4/16/98).

Even if resistance to Cry9C develops, it is unlikely to significantly impact current agricultural practices used to control ECB or other potentially susceptible pests of corn (even if these pests move to other crops). While the principal host for ECB is corn, it will feed on many other plants and has achieved pest status on potato (Kennedy and Anderson, 1980), bell pepper (Welty, 1995), and cotton (Ellsworth and Bradley, 1992). The *Bt* Cry9C protein has never before been used commercially for insect control either as a formulation for use on corn or other crops or as expressed in transgenic plants (Petition, pg. 46). Should Cry9C-resistant insect populations evolve, they are unlikely to exhibit cross-resistance to those other *Bt* toxins currently commercialized in formulations or expressed in plants that are most often used in corn, because they are not likely to share the same receptors. This statement is supported by the receptor binding studies conducted with Cry9C (Lambert et al., 1996) and the fact that studies of Diamondback moth *(Plutella xylostella)* populations that have developed resistance in the field to *Bt* sprays containing Cry1A proteins demonstrate that cross-resistance only extends to insect control proteins that bind to the Cry1A binding site, i.e. Cry1Aa, Cry1Ab, Cry1Ac, and Cry1F (Tabashnik et al., 1993, 1994, Ferre et al., 1991, Tang et al., 1996, Granero et al., 1996). While some lepidopterans, *Spodoptera exigua* and *P. xylostella*, appear to have receptors that are recognized by both Cry9C and Cry1Cax (Lambert et al., 1996), the only commercial insecticides that contain the Cry1C class

also contain other Cry proteins to which the target pests are sensitive (Sue MacIntosh and Sharlene Matten, personal communication to S. Koehler, 4/16/98). Furthermore, Cry1Cax alone is not toxic to the ECB or *Agrotis* spp. (Lambert et al., 1996). Cross-resistance due to other mechanisms, such as gross changes in feeding behavior or preferences, can not be ruled out.

APHIS concludes that development of resistance to insecticides is a potential issue associated with their use. However, in this respect, cultivation of CBH-351 corn should pose no greater effects on the control of insects in corn and other crops than the currently practiced methods of ECB control; i.e., the use of ECB-tolerant corn cultivars, including the other *Bt* transgenic corn which have received a determination of non-regulated status from APHIS, and the application of chemical and biologically-based insecticides.

Multiple herbicide families (e.g. triazines, dicamba, 2,4-D, and sulfonylureas) are often necessary to control the many grassy and broadleaf weeds present in corn acreage planted in the United States. These applications may occur preplant, pre-emergence and post-emergence to the crop depending on the herbicide chemistry and specific weeds targeted. Multiple post-emergent applications are not widely used due, in part, to potential crop injury and concerns regarding residual activity on other crops planted in rotation with corn. AgrEvo has stated that CBH-351 corn exhibits tolerance to glufosinate ammonium herbicides at concentrations that provide effective weed control and excellent crop safety (Petition, pg. 46 and Annex 8).

CBH-351 corn, along with glufosinate ammonium herbicides, is expected to positively impact current agricultural practices used for weed control in a manner similar to other glufosinate-tolerant corn transformation events which have received a determination of non-regulated status by APHIS, that is by 1) offering growers a broad spectrum, post-emergent weed control system; 2) providing the opportunity to continue to move away from pre-emergent and residually active compounds; 3) providing a new herbicidal mode of action in corn that allows for improved management of weeds which may have developed resistance to herbicides with different modes of action; and 4) decreasing cultivation needs and increasing the amount of no-till acres. Liberty® is currently registered by the U.S. EPA for use only on LibertyLink (glufosinate-tolerant) crops - field corn and soybeans. Volunteers of CBH-351 can be easily controlled by selective mechanical or manual weed removal or by the use of herbicides with active ingredients other than glufosinate ammonium as discussed (Determination Section IV B).

V. CONCLUSION

APHIS has determined that Transformation Event CBH-351 corn that has been field tested under APHIS authority, will no longer be considered a regulated article under

regulations at 7 CFR Part 340. Permits or notifications acknowledged under those regulations will no longer be required from APHIS for field testing, importation, or interstate movement of Transformation Event CBH-351 corn or its progeny. Importation of Transformation Event CBH-351 corn seeds is still, however, subject to the restrictions found in the Foreign Quarantine Notice regulations at 7 CFR Part 319 just as applies to any other importation of corn seeds. This determination has been made based on data collected from these approved field trials, laboratory analyses and literature references presented herein which demonstrate the following:

1. Transformation Event CBH-351 corn exhibits no plant pathogenic properties. Although DNA from plant pathogens were used in their development, these corn plants are not infected by these organisms nor can these plants incite disease in other plants.

2. Transformation Event CBH-351 corn is no more likely to become a weed than insect resistant, herbicide-tolerant corn which could potentially be developed by traditional breeding techniques. Corn is not a serious, principal or common weed pest in the U.S., and there is no reason to believe that resistance to certain lepidopteran insects and tolerance to glufosinate herbicides would enable corn to become a weed pest.

3. Multiple barriers insure that gene introgression from Transformation Event CBH-351 corn into wild plants in the United States and its territories is extremely unlikely, and such rare events should not increase the weediness potential of any resulting progeny.

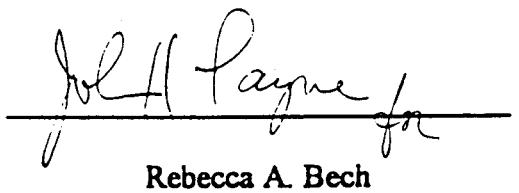
4. Transformation Event CBH-351 corn is substantially equivalent in kernel composition, quality and other characteristics to nontransgenic corn and should have no adverse impacts on raw or processed agricultural commodities.

5. Transformation Event CBH-351 corn exhibits no significant potential to harm organisms beneficial to the agricultural ecosystem and will not affect threatened or endangered species.

6. Compared to current corn cultivation practices, cultivation of Transformation Event CBH-351 corn should not reduce the ability to control insects or weeds in corn or other crops.

APHIS has also concluded that there may be new varieties bred from Transformation Event CBH-351 corn; however, if such varieties are developed they are unlikely to exhibit new plant pest properties, i.e., properties substantially different from any

observed for Transformation Event CBH-351 corn already field tested, or those observed for corn developed from traditional breeding.



Rebecca A. Bech
Assistant Director, Scientific Services
Plant Protection and Quarantine
Animal and Plant Health Inspection Service
U.S. Department of Agriculture
Date MAY 8 1998

VI. LITERATURE CITED

Baker, H. G. 1965. Characteristics and modes of origin of weeds, pp. 147-168. *In:* The genetics of colonizing species, Baker, H. G., and Stebbins, G. L. (eds.). Academic Press, NY.

Bayer, E., Gugel, K.H., Hagele, K., Hagenmaier, H., Jessipow, S., König, W.A., Zähner, H. 1972. Stofwechproduckte von Mikroorganismen. Phosphinothricin und Phosphinothricyl-alanyl-alanin. Helvetica Chimica Acta 55: 224-239.

Chiang, H.C., Wilsoxson, R.D. 1961. Interactions of the European corn borer and stalk rot in corn. J. Econ. Entomol. 54:850-852.

Christensen, J.J., Schneider, C.L. 1950. European corn borer *(Pyrausta nubilalis* Hbn.) in relation to shank, stalk, and ear rots of corn. Phytopathology 40:284-291.

DeBlock, M., Botterman, J., Vandewiele, M., Dockx, J., Thoen,C., Gosselé, V., Rao Movva, N., Thompson, C., Van Montagu, M., Leemans, J. 1987. Engineering herbicide resistance in plants by expression of a detoxifying enzyme. EMBO 6:2513-2518.

Doebley, J. 1990a. Molecular evidence for gene flow among *Zea* species. BioScience 40:443-448.

Doebley, J. 1990b. Molecular systematics of *Zea* (Gramineae). Maydica 35(2):143-50.

Ellsworth, P.C., and Bradley Jr., J.R., 1992. Comparative damage potential and feeding dynamics of the European corn borer (Lepidoptera:Pyralidae) and cotton bollworm (Lepidoptera:Noctuidae) on cotton bolls. J. Econ. Entomol. 85:402-410.

Ferré, J., Real, M.D., Van Rie, J., Jansens, S., Peferoen, M., 1991. Resistance to the *Bacillus thuringiensis* bioinsecticide in a field population of *Plutella xylostella* is due to a change in a midgut membrane receptor. Proceeding of the National Academy of Sciences, USA, 88:5119-5123

Foundation for Nutritional Advancement. 1996. Executive Summary. Antibiotic Resistance Via the Food Chain, Case Study: Ampicillin. Conference held September 23-24, 1996 at Tufts University, Talloires, France. Foundation for Nutritional Advancement, Boston, MA.

Free, J.B. 1970. Insect Pollination of Crops. Academic Press Inc., London, pp. 544.