Notices

Federal Register
Vol. 60, No. 119
Wednesday, June 21, 1995

Animal and Plant Health Inspection Service

[Docket No. 95-007-2]

Availability of Determination of Nonregulated Status for Genetically Engineered Corn

AGENCY: Animal and Plant Health Inspection Service, USDA.

ACTION: Notice.

SUMMARY: We are advising the public of our determination that Ciba Seeds' corn designated as Event 176 Corn that has been genetically engineered for insect resistance 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 Ciba Seeds 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 Ciba Seeds 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 17, 1995.

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 asked to call in advance of visiting at (202) 690–2817.

FOR FURTHER INFORMATION CONTACT: Dr. Ved Malik, Biotechnologist, Biotechnology Permits, BBEP, APHIS, 4700 River Road Unit 147, Riverdale, MD 20737–1237; (301) 734–7612. To obtain a copy of the determination or the environmental assessment and finding of no significant impact, contact Ms. Kay Peterson at (301) 734–7612.

SUPPLEMENTARY INFORMATION:

Background

On November 15, 1994, the Animal and Plant Health Inspection Service (APHIS) received a petition (APHIS Petition No. 94–319–01p) from Ciba Seeds of Research Triangle Park, NC, seeking a determination that corn designated as Event 176 Corn that has been genetically engineered for insect resistance does not present a plant pest risk and, therefore, is not a regulated article under APHIS' regulations in 7 CFR part 340.

On February 21, 1995, APHIS published a notice in the Federal Register (60 FR 9656–9657, Docket No. 95–007–1) announcing receipt of the Ciba Seeds petition and announcing that the petition 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 and food products derived from it. In the notice, APHIS solicited written comments from the public as to whether the subject corn posed a plant pest risk. The comments were to have been received by APHIS on or before April 24, 1995.

APHIS received 37 comments on the Ciba Seeds petition. Comments were received from farm-related businesses, universities, national and State associations, farmers cooperatives, farmers, individuals, a cooperative extension research center, and a member of the U.S. House of Representatives. Thirty-five commenters either expressed support for the Event

176 Corn petition for nonregulated status or endorsed the concept of an insect-resistant corn variety without specific reference to the petition. Two of the 37 commenters expressed reservations about a determination in favor of the subject petition based on their concerns about resistance management. APHIS has provided a summary and discussion of the comments in the determination document, which is available upon request from the individual listed under **FOR FURTHER INFORMATION CONTACT.**

Analysis

Ciba Seeds' Event 176 Corn has been genetically engineered to express an insect control protein representing a truncated form of the CryIA(b) protein that occurs naturally in *Bacillus thuringiensis* subsp. *kurstaki* (*Btk*), a common gram-positive soil bacterium. *Btk* proteins are very effective against certain lepidopteran insects, including European corn borer (ECB). Event 176 Corn has been modified to produce the CryIA(b) protein in green tissues and pollen cells. During field tests of Event 176 Corn, ECB infestations were significantly reduced as compared to the nontransgenic control plants.

The subject corn has been considered a regulated article under APHIS' regulations in 7 CFR part 340 because it contains certain gene sequences derived from plant-pathogenic sources. However, evaluation of field data reports from field tests of the subject corn conducted since 1992 indicates that there were no deleterious effects on plants, nontarget organisms, or the environment as a result of the subject corn plants' release into the environment.

Determination

Based on its analysis of the data submitted by Ciba Seeds and a review of other scientific data, comments received from the public, and field tests of the subject corn, APHIS has determined that Event 176 Corn: (1) Exhibits no plant pathogenic properties; (2) is no more likely to become a weed than lepidopteran-insect-resistant corn developed through traditional breeding techniques; (3) is unlikely to increase the weediness potential of any other cultivated plant or native wild species with which it can interbreed; (4) should not cause damage to raw or processed agricultural commodities; (5) is unlikely to harm organisms beneficial to the agricultural ecosystem; and (6) when cultivated, should not reduce the ability to control insects in corn and other crops. APHIS has also concluded that there is a reasonable certainty that new varieties developed from Event 176 Corn will not exhibit new plant pest properties, i.e., properties substantially different from any observed in the field tested Event 176 Corn, or those observed in corn in traditional breeding programs.

The effect of this determination is that insect-resistant corn designated as Event 176 Corn is no longer considered a regulated article under APHIS' regulations in 7 CFR part 340. Therefore, the permit and notification 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, the importation of the subject corn or seeds capable of propagation is 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 (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 the subject corn 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 13th day of June 1995.

Lonnie J. King,

Acting Administrator, Animal and Plant Health Inspection Service.

[FR Doc. 95–15112 Filed 6–20–95; 8:45 am]

BILLING CODE 3410–34–P

USDA/APHIS Petition 94-319-01 for Determination of Nonregulated Status for Event 176 Corn

Environmental Assessment and
Finding of No Significant Impact

May 1995

The Animal and Plant Health Inspection Service (APHIS) of the U. S. Department of Agriculture has conducted an environmental assessment before issuing a determination of nonregulated status for a genetically engineered corn called event 176 corn. APHIS received a petition from the Ciba Seeds regarding the status of the event 176 corn as a regulated article under APHIS regulations at 7 CFR Part 340. APHIS has conducted an extensive review of the petition and supporting documentation, also 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 event 176 corn shall no longer be a regulated article.

John H. Payne

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Acting Director
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Animal and Plant Health Inspection Service
U.S. Department of Agriculture
Date: MAY 17 1995

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I. <u>SUMMARY</u>

The Animal and Plant Health Inspection Service (APHIS), United States Department of Agriculture (USDA), has prepared an Environmental Assessment (EA) before deciding on the regulated status of a genetically engineered line of lepidopteran (caterpillar) insect resistant corn designated hereafter as event 176 corn. The developer of event 176 corn, the Ciba Seeds petitioned APHIS requesting a determination on the regulated status of event 176 corn that has been a regulated article under APHIS regulations. Interstate movements and field tests of event 176 corn have been conducted under permits issued by or notifications acknowledged by APHIS. Ciba has petitioned APHIS for a determination that event 176 corn does not present a plant pest risk and should therefore no longer be a regulated article under the APHIS regulations found at 7 CFR Part 340.

The event 176 corn has been developed in an effort to protect the corn plants against the feeding damage caused by the larvae of European corn borer (*Ostrinia nubilalis* (Hubner)). Genes encoding insecticidal crystal proteins from *Bacillus thuringiensis* subsp. *kurstaki* have been inserted into the corn chromosome. A selectable genetic marker encoding phosphinothricin acetyltransferase also been introduced into the corn chromosome but only to facilitate selection of transformed cells in the laboratory. The genes were introduced via a well-characterized procedure that results in direct introduction of genes into plant genomes.

EAs were prepared before granting the permits for event 176 corn field trials. Previous EAs addressed questions pertinent to plant pest risk issues concerning the conduct of field trials under physical and reproductive confinement, but they do not address several issues that are of relevance to the unconfined growth of event 176 corn. With respect to these new issues, APHIS concludes the following:

1. Event 176 Corn exhibits no plant pathogenic properties. Although pathogenic organisms were used in their development, these corn plants are not infected by these organisms nor can these plants incite disease in other plants.

2. Event 176 Corn is no more likely to become a weed than insect-resistant 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 insects would enable corn to become weed pests.

3. Multiple barriers insure that gene introgression from Event 176 Corn into wild or cultivated sexually-compatible plants is extremely unlikely, and such rare events should not increase the weediness potential of any resulting progeny or adversely impact biodiversity.

4. Seeds of Event 176 Corn are substantially equivalent in composition, quality and other characteristics to nontransgenic corn

and should have no adverse impacts on raw or processed agricultural commodities.

5. Event 176 Corn exhibits no significant potential to either harm organisms beneficial to the agricultural ecosystem or to impair the ability of farmers to control nontarget insect pests.

6. Cultivation of Event 176 Corn should not reduce the ability to control insects in corn and other crops.

Therefore, after a review of the available evidence, APHIS believes that event 176 corn will be just as safe to grow as traditionally-bred lepidopteran insect resistant 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 event 176 corn were no longer considered a regulated article under regulations at 7 CFR Part 340.

II. BACKGROUND

Development of event 176 corn. Ciba has submitted a "Petition for Determination of Non-regulated Status" to the USDA, APHIS for corn plants that contain a gene that protects the corn plants against the feeding damage caused by the larvae of European corn borer. Ciba requested a determination from APHIS that the event 176 corn, and any progeny derived from hybrid crosses between this line and other non-transformed corn varieties, no longer be considered regulated articles under 7 CFR Part 340.

European corn borer (ECB) damage to corn plants results in stalk lodging, dropped ears, and damaged grain. Yield reductions due to ECB infestations are estimated to exceed $50 million annually in the State of Illinois alone. *B. thuringiensis* subsp. *kurstaki* produces a family of related toxins (delta-endotoxin) that when ingested by susceptible lepidopteran insects result in their death. These toxins are produced in crystalline structure in and during the bacterial spore formation. Preparations of *B. thuringiensis* containing delta-endotoxins are used foliar applied biopesticides. However, they are not routinely effective against ECB because the insect feeds inside the plants where the foliar applied biopesticide cannot reach. Ciba Seeds has modified the corn plant to produce in green tissues and pollen cells a specific delta-endotoxin, called *cryIA(b)*. During field testing of event 176 corn that express *cryIA(b)*, ECB infestations were significantly reduced as compared to the nontransgenic control plants. The expression of the two copies of the *cryIA(b)* genes are under the control either a pollen-specific promoter derived from calcium-dependent protein kinase or green tissue-specific promoter phosphoenolpyruvate carboxylase. Both promoters were isolated from corn plants. The termination sequences for both of genes was from cauliflower mosaic virus (CaMV), a known plant pest.

Event 176 corn has also been transformed with the gene from *Streptomyces hygroscopicus* that encodes the enzyme phosphinothricin acetyltransferase and serves as a selectable marker enabling identification of the transformed plant cells. This gene is fused to 35S promoter sequence and termination sequence from CaMV.

These two genes were introduced into event 176 corn via microprojectile bombardment transformation. This is a well-characterized procedure that has been used for nearly a decade for introducing various genes of interest directly into plant genomes.

Event 176 corn has been field tested since 1992 in the major corn growing regions of the United States under permits and acknowledgements of notifications by APHIS (USDA No. 92-042-01, 92-127-01, 92-140-01, 93-014-01, 93-120-08, 93-363-01, 94-076-10, and 94-347-05). Event 176 corn has been evaluated extensively in laboratory, greenhouse, and field experiments to confirm that it exhibits the desired agronomic characteristics and does not pose a plant pest risk. Although the field tests of event 176 corn have been conducted in agricultural settings, the permit conditions and acknowledgement of notifications for the tests have stipulated physical and reproductive confinement from other plants.

APHIS Regulatory Authority. APHIS regulations at 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. Event 176 corn described in the Ciba petition has been considered a regulated article because noncoding DNA regulatory sequences and portions of the plasmid vector are 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, the Agency can grant the petition in whole or in part. Therefore, APHIS permits would no longer be required for field testing, importation, or interstate movement of that article or its progeny.

Environmental Protection Agency (EPA) and Food and Drug Administration (FDA) Regulatory Authority. Event 176 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. Accordingly, Ciba has submitted to EPA an application to register this plant-pesticide, i.e., *cry IA* gene and its controlling sequences in event 176 corn. On December 8, 1993, EPA announced receipt of this application (EPA File Symbol 524-UTU) in the Federal Register (58 FR 64582-64583). The EPA has not yet announced its final decision on this registration 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 practice, it will not generally 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. Ciba has submitted to the EPA a pesticide petition (PP 3F4273) proposing to amend 40 CFR part 180 to establish a tolerance exemption for residues of the plant pesticide active ingredient *Btk* CPB control protein as expressed in plant cells. On December 8, 1993, EPA announced receipt of this petition [58 FR 64583-64584]. The EPA has not yet announced its decision on this petition.

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.

III. PURPOSE AND NEED

APHIS has prepared this EA before making a determination on the status of event 176 corn as a regulated article under APHIS regulations. The developer of event 176 corn, Ciba Seeds, submitted a petition to USDA, APHIS requesting that APHIS make a determination that event 176 corn shall no longer be considered a regulated article under 7 CFR Part 340.

This EA was prepared in compliance with the National Environmental Policy Act (NEPA) of 1969 (40 CFR 1500-1508) and the pursuant implementing regulations published by the Council on Environmental

Quality (42 USC 4331 *et seq.*; 40 CFR 1500-1508; 7 CFR Part 1b; 44 FR 50381-50384; and 44 FR 51272-51274).

IV. ALTERNATIVES

A. No Action.

Under the Federal "no action" alternative, APHIS would not come to a determination that event 176 corn is no longer a regulated article under the regulations at 7 CFR Part 340. Permits from APHIS would still be required for introductions of event 176 corn. APHIS might choose this alternative if there were insufficient evidence to demonstrate the lack of plant pest risk from uncontained cultivation of event 176 corn.

B. Determination that event 176 corn is no longer a regulated article.

Under this alternative, event 176 corn would no longer be a regulated article under the regulations at 7 CFR Part 340. Permits from APHIS would no longer be required for introductions of event 176 corn. A basis for this determination would include a "Finding of No Significant Impact" under the National Environmental Policy Act of 1969 (42 USC 4331 *et seq.*; 40 CFR 1500-1508; 7 CFR Part 1b; 44 FR 50381-50384; and 44 FR 51272-51274).

V. AFFECTED ENVIRONMENT AND POTENTIAL ENVIRONMENTAL IMPACTS

This EA addresses potential environmental impacts from a determination that event 176 corn should no longer be considered a regulated article under APHIS regulations at 7 CFR Part 340. Previous EAs prepared by APHIS with the issuance of permits for field tests of event 176 corn have addressed various attributes of this corn. This EA discusses the genetic modification, and the potential environmental impacts that might be associated with the unconfined cultivation of event 176 corn.

Additional technical information is included in the determination document appended to this EA, and incorporated by reference. This includes detailed discussions of the biology of corn, the genetic components used in the construction of event 176 corn, and the analyses that lead APHIS to conclude that event 176 corn has no potential to pose plant pest risks.

A. Potential impacts based on increased weediness of event 176 corn relative to traditionally bred insect resistant corn

Although various definitions of the term "weed" have been proposed in the scientific literature, the salient point is that a plant can be considered a weed when it is growing where humans do not want it

(Baker 1965; de Wet and Harlan 1975; Muenscher 1980). Baker (1965) lists 12 common attributes that can be used to assess the likelihood that a plant species will behave as a weed. 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.

The cultivated corn is not considered a weed pest and is unlikely to become a weed pest. Corn is considered a highly inbred, well-characterized crop plant that is not persistent in undisturbed environments without human intervention. Although corn volunteers are not uncommon, they are easily controlled using herbicides or mechanical means. Corn also possess few of the characteristics of plants that are notably successful weeds (e.g., it does not produce abundant, long-lived seed; it does not propagate vegetatively; it does not compete well with other plant species in the environment).

Furthermore, corn has been grown for centuries throughout the world without any reports that it is a serious weed pest. 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).

The parent plant of the event 176 corn is an agricultural crop plant that exhibits no appreciable weedy characteristics. The relevant introduced trait, lepidopteran insect resistance, is unlikely to increase weediness of event 176 corn. There is no indication that the presence of a *cryIA(b)* gene in resulting event 176 corn will convert it into a weed. The corn plants have also been transformed with a phosphinothricin acetyltransferase, which confers resistance to the herbicide glufosinate. The gene has no involvement in plant disease or damage. Also, its use does not result in the presence of the herbicide in corn and does not imply that glufosinate will be used in the cultivation of the corn. No other attributes of event 176 corn suggest that it be any more "weedy" than the present corn cultivars that are the result of traditional breeding. The event 176 corn has retained the agronomic characteristics of the parental corn. Ciba has provided data regarding seed germination rates, yield characteristics, disease and pest susceptibilities, compositional analyses, and numerous other test reported in chapter 9 and 10 in the Ciba application that support APHIS' conclusion that event 176 corn is just as safe to grow as any other insect resistant corn.

B. Potential impacts on the sexually-compatible relatives of corn arising from pollination by event 176 corn

Zea is a genus of the family Gramineae (the grass family) that consists of some 4 species: *Z. mays*, cultivated corn and teosinte; *Z. diploperennis*, diploperennial teosinte; *Z. luxurians*; and *Z. perennis*, a perennial teosinte. Of the four species of *Zea*, only *Z. mays* is common in the United States. It is known only from cultivation; it occasionally is spontaneous in abandoned fields or roadsides, but is incapable of sustained reproduction outside of cultivation (Gould 1968). The other species are occasional university or experiment station research subjects. *Z. perennis* is reported as established from James Island, South Carolina (Hitchcock and Chase 1951).

The closest relative to *Zea* is *Tripsacum*, a genus of seven species, three of which occur in the United States (Gould 1968). *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 resulting seeds are sterile (Galinat 1988).

Cultivated corn is presumed to have been transformed from teosinte, *Z. mays* subsp. *mexicana* more than 8000 years ago. During this transformation, cultivated corn gained several valuable agronomic traits, but lost the ability to survive in the wild. Teosinte, however, remains a successful wild grass in Mexico and Guatemala. Despite some confusion over proper taxonomic groupings of the non-cultivated members of *Zea*, wild members maintain a successful array of annual or perennial plants with visible chromosomal peculiarities and ploidy levels, and many adaptive macroscopic phenotypes. Cultivated corn and the wild members of diploid and tetraploid *Zea* can be crossed to produce fertile F_1 hybrids. Nonetheless, in the wild, introgressive hybridization does not occur because of differences in flowering time, geographic separation, block inheritance, developmental morphology and timing of the reproductive structures, dissemination, and dormancy (Galinat 1988).

The second major transformation of cultivated corn occurred in the United States in the twentieth century, and particularly since the 1930's. This transformation occurred through inbred lines for hybrid seed production, and by other methods. Almost all corn grown in the United States now comes from hybrid seed that is obtained every planting season from private enterprises; the older open-pollinated varieties are virtually unknown in commerce (Hallauer et al. 1988). This transformation has resulted in more uniform commercial plants with superior agronomic characteristics, and has contributed to the six-fold increase in per acre yields in the last sixty years.

Our analysis of the biology of cultivated lepidopteran insect resistant corn and its relatives leads us to predict that the environmental impacts of cultivation of event 176 corn anywhere in the world would be no different from such impacts attributable to similar varieties produced with traditional breeding techniques. The species *Z.mays* is native to Mexico and Central America. Non-cultivated varieties of *Zea* sp. have coexisted and co-evolved in the Americas over millennia. Even if event 176 corn were to be cultivated in agricultural regions around centers of *Zea* diversity, there is no reason to expect impacts from event 176 corn to be significantly different from those arising from the cultivation of any other variety of insect resistant corn.

International traffic in event 176 corn 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 (98 countries as of December 1992). The treaty, now administered by a Secretariat housed with the United Nations Food and Agriculture Organization in Rome, came into force on April 3, 1952. It 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. This includes Mexico, which has in place a regulatory process requiring a full evaluation of event 176 corn before it can be introduced into their environment. The IPPC has also led to the creation of Regional Plant Protection Organizations (RPPOs) such as the North American Plant Protection Organization (NAPPO). Our trading partners will be kept informed of our regulatory decisions through NAPPO and other fora. Our decision in no way prejudices regulatory action in any other country.

It should be noted that all the considerable, existing national and international regulatory authorities and phytosanitary protocols that currently apply to introductions of new lepidopteran insect resistant corn varieties internationally apply equally to those covered by this analysis.

C. Potential impacts on nontarget organisms, including beneficial organisms such as bees and earthworms, and threatened or endangered organisms

Consistent with its statutory authority and requirements under NEPA, APHIS evaluated the potential for event 176 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 to 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.

There is no reason to believe that deleterious effects or significant impacts on nontarget organisms, including beneficial organisms, would result from the phosphinothricin acetyltransferase gene used as a selectable marker during development of this line.

1) Potential impact on beneficial and other nontarget organisms.

APHIS evaluated the results of several studies designed to compare the impact on nontarget organisms of event 176 corn and *cryIA(b)* (These experiments are described in the petition, starting on page 180).

a). No effect on survival, immobilization, or sublethal toxicity was seen when a small aquatic insect, *Daphnia magna*, was exposed to pollen collected from event 176 corn as compared to pollen collected from nontransgenic control plants. Pollen was chosen as test material because it is likely to be spread by wind and land in lakes and ponds.

b). Survival rates, signs of toxicity, or loss of weight were not observed in earthworms, *Eisenia foetida*, exposed to leaf tissue from event 176 corn as compared to the control treatments.

c). Lady beetle (*Coleomegilla maculata*) larval development was not affected when reared on pollen collected from event 176 corn plants as compared to pollen from nontransgenic plants.

d). No measurable detrimental effects of ingestion of pollen collected from event 176 corn on larval development of honeybees (*Apis melifera*) was reported.

e). Two lepidopteran insects (fall armyworm and black cutworm) that are not susceptible to native *cryIA(b)* were likewise not affected when feed *cryIA(b)* derived from leaf tissues. Three insects (ECB, corn earworm, and cabbage looper) that are susceptible to native *cryIA(b)* were also susceptible to plant-produced *cryIA(b)*.

f). Results of monitoring of small scale field test indicated no difference in the number of total insects, or the number of specific insect Orders between event 176 corn and control nontransgenic corn plants. However, when comparing to insect populations on plants treated with a common chemical insecticide (permethrin) versus event 176 corn plants, the total numbers of beneficial insects (especially lady beetles) associated with event 176 corn plants were higher.

g), Results from high dose feeding studies of bobwhite quail fed a protein extract enriched in *cryIA(b)* isolated from event 176 corn demonstrated no adverse effects on the bird (Petition, starting on page 180).

h). Ciba presents results from a study using an enriched leaf extract from event 176 corn on the soil arthropod Collembola, *Folsomia candida*. This insect is one of a number of organisms that recycle plant debris in the field. When the concentrations of *cryIA(b)* were 200-fold higher than would be expected under natural conditions a reduction in adult survival and the number of offspring was reduced. This observation was not totally unexpected since a related *B. thuringiensis* subsp. *galleriae* has been reported to kill Collembolla (Atlavintye et al. 1982; Smulevitch et al. 1991; Nakamura, 1994). Postharvest monitoring of field test with event 176 corn plants showed no increase in visible amount of corn debris when compared to nontransgenic plants. Organophosphate insecticides, used to control corn rootworm, reduce mortality of Collembola (Thompson and Gore, 1972). APHIS concludes that there should be no significant adverse effect on Collembola and no increase in corn plant debris as result of the cultivation of event 176 corn plants.

Other invertebrates and all vertebrate organisms, including non-target birds, mammals and humans, are not expected to be affected by the *Btk* insect control protein, because they would not be expected to contain the receptor protein found in the midgut of target insects.

2) Potential impact on threatened and endangered arthropods

The host ranges and habitats of the lepidopteran insect species currently listed or proposed as threatened and endangered in the U.S. were examined to determine if event 176 corn might have an adverse impact on these species. None of these species inhabit corn fields or feed on corn. Most of the endangered species usually occur in specialized habitats. Often the habitat or unique plant that these butterflies or moths require for a successful life cycle is disappearing or threatened by human activities (BBEP-EAD National Endangered Species Database, 1994). For example, Smith's blue butterfly lives in coastal regions around Monterey County, California. Survival of this butterfly is dependent on its larval host food plants, seacliff buckwheat and coast buckwheat. The primary factor limiting the Smith's butterfly is distribution of the two host plants.

APHIS concludes that event 176 corn will not have a significant adverse impact on organisms beneficial to plants or agriculture, nontarget organisms, and will not affect threatened or endangered species.

D. Potential impacts on agricultural and cultivation practices.

There are currently no commercially available corn hybrids that are resistant to ECB. Although chemical insecticides (organophosphates and pyrethroids) and foliar applied *Btk* formulations can be effective against ECB, applications must be applied before the insect bores into the stalk. Repeat applications are often necessary. If commercialized, event 176 corn could offer an important alternative to chemical insecticides. Only about 5 percent of corn acreage in the U.S. is treated with foliar *Btk* products. By the same token, widespread and inappropriate use of either event 176 corn or increased use of foliar microbial *Btk* products can and will most likely accelerate the appearance of ECB populations resistant to the *Btk* insect control protein. The rate with which resistance will develop using either strategy is difficult to predict. The rate depends on many assumptions regarding resistance management strategies, their acceptance and effective implementation by growers, the genetics of ECB resistance to this insecticide, and population and behavioral biology of ECB (Tabashnik 1994a,b; Gould et al. 1994).

The implementation of an active resistance management plan that is scientifically sound and acceptable to growers should delay the onset of resistance and provide alternative strategies and methods for managing or containing resistant populations if they occur. Ciba Seeds has implemented strategies to: (1) develop genes for new insect control proteins, (2) monitor ECB susceptibilities to event 176 corn plants, and (3) conduct and support research relevant to ECB resistance management. Ciba has stated that it is in their best interest to delay resistance. The EPA has stated that they will work with Ciba to develop product labels and informational brochures that are consistent with resistance management, and this should help define the appropriate use of event 176 corn.

If resistant populations persist, insecticides based on the *cryIA(b)* insect control protein would no longer be effective for controlling ECB on corn (ECB is not a serious pest of other crops). To control resistant ECB populations, producers could use currently registered insecticides, possible including new delta-endotoxins.

Based on this analysis, APHIS concludes that there is unlikely to be any significant adverse impact on agricultural practices associated with the appropriate use of event 176 corn. Similar to the consequence of deployment of other insecticides, resistance development in insect pest populations is probable. However, cultivation of ECB-resistant corn plants should pose no greater threat to the ability to control ECB in corn, than that posed by the insecticides already in use.

E. Event 176 corn will not cause damage to processed agricultural commodities.

In APHIS' opinion, the components and processing characteristics of event 176 corn reveal no differences in any component that could have an indirect plant pest effect on any processed plant commodity.

VI. CONCLUSION

APHIS has evaluated information from the scientific literature as well as data submitted by Ciba Seeds that characterized event 176 corn. After careful analysis, APHIS has identified no significant impact to the environment from issuance of a determination that event 176 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. Event 176 Corn exhibits no plant pathogenic properties. Although pathogenic organisms were used in their development, these corn plants are not infected by these organisms nor can these plants incite disease in other plants.

2. Event 176 Corn is no more likely to become a weed than insect-resistant 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 insects would enable corn to become weed pests.

3. Multiple barriers insure that gene introgression from Event 176 Corn into wild or cultivated sexually-compatible plants is extremely unlikely, and such rare events should not increase the weediness potential of any resulting progeny or adversely impact biodiversity.

4. Seeds of Event 176 Corn are substantially equivalent in composition, quality and other characteristics to nontransgenic corn and should have no adverse impacts on raw or processed agricultural commodities.

5. Event 176 Corn exhibits no significant potential to either harm organisms beneficial to the agricultural ecosystem or to impair the ability of farmers to control nontarget insect pests.

6. Cultivation of Event 176 Corn should not reduce the ability to control insects in corn and other crops.

Therefore, after review of the available evidence, APHIS concludes that Event 176 Corn will be just as safe to grow as traditionally-bred, lepidopteran insect resistant corn varieties that are not subject to regulation under 7 CFR Part 340. APHIS concludes that there should be no significant impact on the human environment if Event 176 Corn were no longer considered a regulated article under its regulations (7 CFR Part 340).

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Response to Ciba Seeds Petition for Determination of Nonregulated Status for Insect-Resistant Event 176 Corn

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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 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.

Based on a review of scientific data and literature, the Animal and Plant Health Inspection Service (APHIS) has determined that lepidopteran insect-resistant corn (hereafter referred to as Event 176 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. APHIS made this determination in response to a petition received on November 15, 1994 from Ciba Seeds, a division of Ciba-Geigy Corporation of Research Triangle Park, North Carolina. The petition requested a determination from APHIS that Event 176 Corn does not pose a plant pest risk and therefore, is not a regulated article. On February 21, 1995 APHIS announced receipt of the petition in the *Federal Register* (60 *FR* 9656-9657) and stated that the petition was available for public view. APHIS invited written comments on this proposed action, to be submitted on or before April 24, 1995. As a result of this determination, oversight under those regulations will no longer be required by APHIS for field testing, importation, or interstate movement of Event 176 Corn or its progeny.

Event 176 Corn is genetically engineered with the gene that codes for an insecticidal protein naturally produced by the soil bacterium *Bacillus thuringiensis* subsp. *kurstaki* (*Btk*), and a selectable marker gene encoding phosphinothricin acetyltransferase from *Streptomyces hygroscopicus*. These genes also have accompanying DNA regulatory sequences that modulate their expression. The DNA regulatory sequences were derived from corn and the plant pathogen cauliflower mosaic virus (CaMV).

This determination has been made based on an analysis that revealed that Event 176 Corn plants: 1) exhibit no plant pathogenic properties, 2) are no more likely to become a weed than insect-resistant corn developed by traditional breeding, 3) are unlikely to increase the weediness potential of any other cultivated plant, 4) do not cause damage to processed agricultural commodities, 5) are unlikely to harm other organisms that are beneficial to agriculture or to adversely impact the ability to control nontarget insect pests, and 6) are unlikely to reduce the ability to control insects in corn and other crops. APHIS has also concluded that there is no reason to believe that new corn varieties derived from Event 176 Corn progeny will exhibit new plant pest properties; i.e., properties substantially different from any observed for the Event 176 Corn already field tested, or those observed for corn in traditional breeding programs.

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. 4331 *et seq.*; 40 CFR 1500-1509; 7 CFR Part 1b; 44 FR 50381-50384; and 44 FR 51272-51274). An environmental assessment (EA) was prepared and a preliminary Finding of No Significant Impact (FONSI) was reached by APHIS for the determination that Event 176 Corn is no longer a regulated article under its regulations at 7 CFR Part 340. This decision does not release Event 176 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 insect-resistant corn; (2) a summary of, and response to, 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 insect-resistant 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 this regulation, a genetically engineered organism is deemed a regulated article either 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 APHIS has reason to believe that the genetically engineered organism presents a plant pest risk. The FPPA gives 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 or diseases.

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

An organism is not 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 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 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.

The Event 176 Corn has been considered a "regulated article" for field testing under Part 340 of the regulations in part because certain noncoding regulatory sequences were derived from CaMV, a known plant pest. APHIS believes it prudent to provide assurance before commercialization that organisms such as Event 176 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 Event 176 Corn is not a regulated article is based in part on evidence provided by Ciba Seeds concerning the biological properties of Event 176 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 Part 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 organisms do not present a plant pest risk can be made under this definition, especially when there is evidence that the plant under consideration: 1) exhibit no plant pathogenic properties; 2) are no more likely to become a weed than insect-resistant corn developed by traditional breeding; 3) are unlikely to increase the weediness potential of any other cultivated plant; 4) do not cause damage to processed agricultural commodities; and 5) are unlikely to harm other organisms that are beneficial to agriculture or to adversely impact the ability to control nontarget insect pests. Evidence has been presented by Ciba Seeds 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 Event 176 Corn will exhibit plant pest properties substantially different from any observed for corn in traditional breeding programs, or as seen in the development of Event 176 Corn already field tested. APHIS does anticipate that there will be new corn varieties bred from Event 176 Corn.

B. EPA and FDA regulatory authority

The Event 176 Corn lines are currently subject to regulations administered by the EPA and/or the FDA (described in Section II.C. of the Environmental Assessment) that require registration of pesticides prior to their distribution and sale and establish tolerances for pesticide residues in raw agricultural products. APHIS' decision on the regulatory status of the Event 176 Corn under APHIS' regulations at 7 CFR 340, in no way releases this corn and its progeny from EPA and FDA regulatory oversight.

III. RESPONSE TO COMMENTS

APHIS received 37 comment letters on the subject petition from respondents in the following categories: farm-related businesses (9); universities (9); national and state associations (5); farmers' cooperatives (4); farmers (4); individuals (4); cooperative extension service (1); and a member of the U.S. Congress (1). Thirty-five commenters either expressed support for the Event 176 Corn petition for a determination of nonregulated status or endorsed the concept of an ECB-resistant corn variety without specific reference to the APHIS petition process. Among the general points made by the supporters of the petition were the need for the product because of the economic losses resulting from ECB damage to the nation's corn crop and the anticipated benefits of a reduction in pesticide applications through the use of an lepidopteran-resistant corn variety.

Two of the 37 commenters expressed reservations about a determination in favor of the petition based on their concerns about resistance management. Both of these commenters specifically addressed the need for a significant refuge of non-transgenic corn where Event 176 Corn is grown in order to produce susceptible insects and thus dilute resistance. One of these two commenters urges APHIS to request a commitment for such a refuge from all companies including Ciba Seeds, with an initial level of at least 20% non-transgenic corn on each farm. Besides refuge, there are many other components of resistance management. APHIS recognizes the importance of resistance management. The petitioner is addressing with EPA the issue of insect resistance prior to commercialization.

IV. ANALYSIS OF THE PROPERTIES OF EVENT 176 CORN

A brief discussion of corn biology 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 Linnaeus, known as maize throughout most of the world, and as corn in the United States, is a large, annual, monoecious grass, that is grown for animal feed, silage, vegetable oil, sugar syrups, and other miscellaneous uses. Corn is grown commercially in almost all States of the United States (Jewell, 1989). Corn has been cultivated since the earliest historic times from Peru to central North America. The region of origin is now presumed to be Mexico (Gould, 1968). Dispersal to the Old World is generally deemed to have occurred in the sixteenth and seventeenth centuries (Cobley and Steele, 1976); however, recent evidence indicates that dispersal to India may have occurred prior to the twelfth and thirteenth centuries by unknown means (Johannessen and Parker, 1989).

Zea is a genus of the family Gramineae (Poaceae), commonly known as the grass family. The genus consists of four species: *Z. mays*, cultivated corn and teosinte; *Z. diploperennis* Iltis et al., diploperennial teosinte; *Z. luxurians* (Durieu et Asch.) Bird; and *Z. perennis* (Hitchc.) Reeves et Mangelsd., perennial teosinte. Variations of the species have been assigned to the segregate genus *Euchlaena*, which is not currently recognized, or have been divided into numerous small species within the genus *Zea* (Terrell et al., 1986). Of the four species of *Zea*, only *Z. mays* is common in the United States. It is known only from cultivation; it occasionally is spontaneous in abandoned fields or roadsides, but is incapable of sustained reproduction outside of cultivation (Gould, 1968). The other species are occasional university or experiment station research subjects. *Z. perennis* is reported as established from James Island, South Carolina (Hitchcock and Chase, 1951).

The closest generic relative to *Zea* is *Tripsacum*, a genus of seven species, three of which occur in the United States (Gould, 1968). *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 only with extreme sterility (Galinat, 1988).

Cultivated corn is presumed to have been transformed from teosinte, *Z. mays* subspecies *mexicana* (Schrader) Iltis, more than 8000 years ago. During this transformation, cultivated corn gained several valuable agronomic traits, but lost the ability to survive in the wild. Teosinte, however, remains a successful wild grass in Mexico and Guatemala. Despite some confusion over proper taxonomic groupings of the non-cultivated members of *Zea*, the wild members of the genus, whether annual or perennial exhibit an array of visible chromosomal peculiarities and ploidy levels, and many adaptive macroscopic phenotypes. Cultivated corn and wild diploid and tetraploid members of *Zea* can be crossed to produce fertile F_1 hybrids. Nonetheless, in the wild, introgressive hybridization does not occur because of differences in flowering time, geographic separation, block inheritance, developmental morphology and timing of the reproductive structures, dissemination, and dormancy (Galinat, 1988).

The second major transformation of cultivated corn in the United States started in the 1930's and continues today through the development and use of inbred lines for hybrid seed production. Almost all corn grown in the United States now comes from hybrid seed that is obtained every planting season from private enterprises; the older open-pollinated varieties are virtually unknown in commerce. This transformation has resulted in more uniform commercial plants with superior agronomic characteristics, and has contributed to the six-fold increase in per acre yields in the last sixty years.

A. The introduced genes, their products, and the added regulatory sequences do not present a plant pest risk in Event 176 Corn.

Event 176 Corn was obtained by microprojectile bombardment of propriety corn line, CG00526, an elite inbred of Lancaster parentage with two plasmids. CG00526 was co-transformed with two plasmids (pCIB4431 and pCIB3064) without any additional carrier DNA. Plasmid pCIB4431 contains two different tissue-specific promoters each fused individually to a copy of a synthetic *cryIA(b)* gene. The *cryIA(b)* gene encodes the first 648 amino acids, insecticidal-active (Koziel *et al.*, 1993) truncated product identical to that of *cryIA(b)* gene of *Bacillus thuringiensis* subsp. *kurstaki* strain HD-1 (Dulmage, 1970; Geiser *et al.*, 1986; Hofte and Whiteley, 1989). The truncated synthetic gene accommodates the preferred codon usage for maize (Murray *et al.*, 1989) that allows efficient expression of the *cryIA(b)* gene in plants (Perlak *et al.*, 1991; Koziel *et al.*, 1993). The first promoter is derived from the corn phosphoenolpyruvate carboxylase (PEPC) gene (Hudspeth and Grula 1989). It promotes expression of *cryIA(b)* in green tissue. The second pollen-specific promoter used is derived from a maize calcium-dependent protein kinase (CDPK) gene (Estruch *et al.*, 1994). The combination of PEPC and pollen tissue-specific promoters provides high *cryIA(b)* gene expression in leaves and pollen, where it is most effective in controlling European corn borer. PEPC intron #9 of corn phosphoenolpyruvate carboxylase gene (Hudspeth and Grula, 1989) is located between the *cryIA(b)* structural gene and the 35S terminator. Its presence also increases the expression level of the *cryIA(b)* gene (Luehrsen and Walbot, 1991). This sequence does not code for any protein(s). The 3'untranslated termination sequences (CaMV) 35S from the cauliflower mosaic virus (CaMV) is present adjacent to the PEPC intron #9. Its function is to provide a polyadenylation site, and has been described previously (Rothstein *et al.*, 1987; Sanfacon *et al.*, 1991). This sequence does not code for any protein(s).

The activity of the pollen specific promoter, associated with its native CDPK structural gene in maize, is not modulated by calcium levels in the plant. Rather, the catalytic activity of the mature CDPK protein in maize is affected by calcium levels. Therefore, we do not anticipate that fusion of this promoter sequence to the *cryIA(b)* will manifest in any changes in the calcium requirements of corn.

The second plasmid (pCIB3064) containss the 35S promoter from cauliflower mosaic virus fused to the plant selectable marker gene *bar* from *Streptomyces hygroscopicus*. The *bar* gene encodes the enzyme phosphinothricin acetyltransferase (PAT) which inactivates phosphinothricin, the active component in the herbicide glufosinate (De Block *et al.*, 1987; Thompson *et al.*, 1987) which is not registered for use in corn. The natural substrate for PAT is bialaphos, the naturally produced antibiotic by *Streptomyces hygroscopicus* from which the *bar* gene was isolated. There are no other reported substrates for PAT, nor has PAT demonstrated any activity towards other acetyltransferase substrates (Bell and Charlwood, 1980). Expression of this enzyme allows for selection of transformed plant cells on selective medium, as well as whole-plant tolerance to glufosinate application.

The various sequences outlined above were cloned in plasmid pUC19 to obtain two transformation vectors. The pUC19 also harbors 1) a beta-lactamase gene under the control of a promoter known only to be active in bacterial but not in plant cells. This gene confers ampicillin resistance to the bacterium; 2) the ColE1 origin that permits replication of pUC19 in bacteria; 3) sequence which codes for the alpha-peptide of the beta-galactosidase (*lacZ*) gene. Other than these described regions, there are no proteins encoded by the remaining DNA sequences in pUC19 (Yanisch-Perron *et al.*, 1985).

Southern blot analysis was performed using Event 176 Corn DNA and the *cryIA(b)* DNA as probe. The results indicated that at least two copies of the transformation vector were integrated in Event 176 Corn genome. There was no hybridization of the *cryIA(b)* gene probe to the untransformed control line CG00526. Hybridization with probes corresponding to the *bar* coding region and a CaMV 35S promoter also indicate integration of two copies of those sequences in Event 176 Corn.

B. Expression of the insect control protein in the Event 176 Corn will not likely provide a competitive advantage sufficient to cause these plants to become any more "weedy" than other corn.

APHIS evaluated whether the Event 176 Corn is any more likely to become a successful weed than nontransgenic control corn line CG00526. 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; Muenscher, 1980). Baker 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) (Baker, 1965). He also described the ideal characteristics of weeds as including the following: discontinuous germination and long-lived seeds; rapid seedling growth; rapid growth to reproductive stage; long continuous seed production; self-compatibility, but not obligatory self-pollination or apomyxis; if outcrossing, use of wind or an unspecialized pollinator; high seed output under favorable conditions; germination and seed production under a wide range of environmental conditions; high tolerance or plasticity of climatic and edaphic variation; special adaptations for dispersal; good competitiveness achieved through, for example, allelochemicals or choking growth; and, if perennial, then exhibiting vigorous vegetative reproduction, brittleness either at the lower nodes or of rhizomes or rootstocks, and having the ability to regenerate from severed rootstocks. 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, particularly the line CG00526 variety, 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 and/or Canada in most weed compendiums (Holm et al., 1991; Muenscher, 1955; USDA, 1971; Weed Science Society of America, 1992).

Expression of the insect control protein in the Event 176 Corn will not likely provide a competitive advantage sufficient to cause these to be any more "weedy" than other corn cultivars. None of the characteristics of weeds described by Baker involved resistance or susceptibility to insects. More importantly, in addition to the analysis above, APHIS evaluated field data submitted by Ciba Seeds which specifically demonstrates that Event 176 Corn is no more weedy than the non-modified recipient. Control and Event 176 Corn plants were routinely monitored during field trials for differences in morphological characteristics, disease and insect susceptibility. The Event 176 Corn plants were healthy and exhibited significantly increased yields (9431901 Petition chapter 10).

Based on evaluation of the available literature and data submitted by Ciba Seeds, APHIS concludes that the Event 176 Corn is no more likely than nontransgenic control to present a plant pest risk as a weed.

C. Gene introgression from Event 176 Corn into wild or cultivated sexually-compatible plants is unlikely, and such rare events should not increase the weediness potential of resulting progeny or adversely impact biodiversity.

APHIS evaluated the potential for gene flow from Event 176 Corn to other cultivated and wild relatives. Then two potential impacts that might result from this sexual transfer of genes were evaluated: first, that the traits from Event 176 Corn might cause free-living relatives to become "weedier", and second, that the transfer of genes might cause population changes that would lead to reduced genetic diversity. The phosphinothricin resistance trait used as a selectable marker in the Event 176 Corn was considered not to pose a hazard in this analysis. Glufosinate is not registered as a pesticide for use in corn and exerts no selection pressure for this trait in nature.

Potential for gene introgression into other corn cultivars via cross pollination is possible. If pollen of the Event 176 Corn can be transferred by wind to any receptive corn stigma within the 30 minute period of pollen viability, cross-pollination could occur. This potential transfer becomes more unlikely as distance increases from the transgenic plants, and from a practical standpoint becomes increasingly unlikely at distances much beyond the foundation seed isolation distance of 660 feet. Farmers purchase hybrid corn seed for planting from a commercial source. If pollen of Event 176 Corn fertilizes the corn fields of a farmer, this corn seed will likely be crushed for use and would not likely be used as seed. Therefore, fertilization of nontransgenic corn by pollen from Event 176 Corn in fields grown for sale as food or feed will not result in dissemination of the trait in to seed populations used for replanting. Moreover, cobs resulting from such fertilization will not themselves express the introduced genetic material.

As stated in Section IV., Ciba Seeds reported no obvious differences in the flowering of Event 176 Corn compared to the nontransgenic parent. There is no reason to believe that the genetic construct introduced during the transformation event would have any effect on the reproductive biology of the Event 176 Corn, unless the insertion event interrupted a genetic locus critical for the normal reproductive function.

Breeder seed is usually derived from self-pollinated seed at the F_8 to F_{10} generation of inbreeding (Wych, 1988). A high degree of self-pollination is ensured by planting well isolated blocks that virtually guarantee natural random sib mating. Minimum isolation distances for foundation seed are one-eighth mile (660 feet) from the nearest contaminating source. Other safeguards, such as physical barriers or unharvested border rows, can further reduce the possibility of contamination. Fields are preferred that have not been recently planted in corn. This is to minimize the appearance of volunteer corn from a previous season. Corn appears as a volunteer in some fields and roadsides, but it never has been able to establish itself outside of cultivation (Gould et al, 1994).

D. Use of Event 176 Corn should have no more adverse impacts on raw or processed agricultural commodities than the parent corn.

During extensive field testing, the Event 176 Corn exhibited the typical agronomic characteristics of the recipient, with the exception of the desired event 176 phenotype conferred by the *Btk* insect control protein. In APHIS' opinion, the components, quality and processing characteristics of Event 176 Corn reveal no differences that could have an indirect plant pest effect on any raw or processed plant commodity. None of the sequences introduced is associated with any disease specific property of the donor organism from which it was derived, and Event 176 Corn exhibit no plant pest characteristics.

E. Event 176 Corn exhibits no significant potential to either harm organisms beneficial to the agricultural ecosystem, to harm threatened or endangered organisms or to have an adverse impact on the ability to control nontarget insect pests.

Organisms beneficial to the agricultural ecosystem: Consistent with its statutory authority and requirements under NEPA, APHIS evaluated the potential for Event 176 Corn plants and plant products and the *Btk* insect control protein to have damaging or toxic effects directly or indirectly on nontarget organisms, particularly those that are recognized as beneficial to agriculture. APHIS also considered potential impacts on other nontarget pests, since such impacts could have an impact on the potential for changes in agricultural practices. There is no reason to believe that the *bar* protein conferring phosphinothricin resistance in the Event 176 Corn plants as a selectable marker for transformation would have deleterious effects or significant impacts on nontarget organisms, including beneficial organisms. Glufosinate herbicide, the target of the *bar* gene product is not registered for use in corn. There have been no reports of toxic effects on such organisms in the many field trials conducted with many different plants expressing this selectable marker.

Ciba seeds examined the toxicity of the Bt maize CryIA(b) protein to nontarget organisms. Two primary test materials were used in these studies: (1) a CryIA(b)-enriched leaf protein preparation (referred to as *Bt* maize protein), obtained by extracting *Bt* maize leaves, enriching the protein for the CryIA(b) fraction, and lyophilizing the material to yield a fine protein powder, and (2) pollen collected from *Bt* maize plants (referred to as *Bt* pollen) that were homozygous for the transgenes. In addition, for certain tests comparing the activity of *Bt* maize protein and native CryIA(b), a cell paste containing the CryIA(b) crystal protein produced by fermentation of *B.t.k.* strain HD1-9 was used. This was referred to as native CryIA(b). The specific material selected for a study was based on the most likely route of exposure for the organism being tested (e.g., aquatic organisms were exposed to pollen because pollen is the most likely part of a corn plant expressing CryIA(b) to enter an aquatic environment).

Ciba Seeds conducted a small plot field study in Bloomington, IL during the summer of 1993 to evaluate the impact of maize expressing the CryIA(b) endotoxin on associated populations of insects. The study focused on beneficial predators and parasites in the orders Diptera, Hymenoptera, and Coleoptera (Coccinellid family) as well as Homopterans, which represent an important food source for beneficial predators. Insect populations in transgenic hybrid maize plots were compared to populations in isogenic hybrid maize and wild type maize plots. The study also evaluated the impact of a conventional chemical insecticide, permethrin, on insect populations in maize. Results of the monitoring study indicated no difference in the number of total insects or the numbers in specific Orders between the transgenic maize plots and either the isogenic or wild type control maize plots. There was no shift in the taxonomic distribution of insects associated with the *Bt* maize compared to the control maize. In contrast, treatment with permethrin, an insecticide used to control ECB, had significant effects on the total numbers of insects and on the numbers within specific groups compared to the untreated plots. The beneficial lady beetle predators (coccinellids) were particularly susceptible to permethrin. Coccinellids, dipterans, and hymenopterans represent the majority of beneficial predators and parasites associated with maize. The results suggest that expression of CryIA(b) in Event 176 Corn maize should not adversely effect insects in these groups. A higher number of beneficial insects including lady beetles were observed in the Event 176 Corn field compared to the field that was treated by permethrin. Only a specific group of lepidopterans should be affected by Event 176 Corn, with the majority of potential prey species remaining unaffected.

Threatened or endangered organisms: An avian acute oral toxicity study using *Bt* maize protein and isogenic maize protein on 8-week-old bobwhite quail produced no mortalities in any test or control group. There were no remarkable necropsy findings in any test or control birds. No adverse effects on body weight or feed consumption were noted for the birds dosed with CryIA(b) protein when compared to either the isogenic or negative control birds. Another theoretically possible, though improbable, route to impact on endangered species in other taxonomic groups, such as birds, is through changes in food supply. However, European corn borer (ECB), the target insect for CryIA(b) in maize, is not a significant food source for birds. As a cryptic insect, ECB larvae bore into the plant and become inaccessible to predators. Although beneficial lady beetles feed on ECB eggs, the potentially reduced ECB populations in areas where *Bt* corn is grown should not impact this predator species because it has many other food sources. Also, as described below, Cry1A(b) protein is nontoxic to lady beetles.

Potential exposure of endangered aquatic organisms would primarily be through pollen blown into the water. Pollen obtained from Event 176 Corn did not adversely effect the aquatic invertebrate *Daphnia magna*, or larval development of lady beetles or honey bees. No effect on survival or signs of toxicity were noted in the earthworms exposed to transgenic protein derived from Event 176 Corn. Invertebrates, such as earthworms, and all vertebrate organisms including nontarget birds, mammals and humans, are not expected to be affected by the *Btk* insect control protein, because they would not be expected to contain the receptor for *Btk* insect control protein found in the midgut of target insects.

Endangered lepidopterans may conceivably be sensitive to CryIA(b) protein, given that the protein is selectively toxic to certain lepidopteran species. Endangered lepidopterans include several species of moths and butterflies: the El Segundo blue butterfly, the primrose sphinx moth, Lange's metalmark butterfly, the Lotis blue butterfly, the Oregon silverspot butterfly, the San Bruno elfin butterfly, Schaus' swallowtail butterfly, and Smith's blue butterfly. These species are not found in areas where corn is commonly grown. Corn is not among the host plants for these lepidopterans. Unlike the exposure scenarios typical of conventional or microbial insecticides, an organism must actually consume maize tissue to receive any exposure to CryIA(b). Therefore, we conclude that threatened and endangered organisms will not be affected as a result of Event 176 Corn.

Host-range comparisons have not indicated any change in range of species susceptible to maize CryIA(b) compared to native CryIA(b). In its effects on insects, Event 176 Corn is similar to the microbial insecticidal preparations that are already commercially sold. Both field testing and laboratory testing of maize CryIA(b) have indicated that nontarget beneficial insects are not likely to be affected by maize CryIA(b), so it is not likely that endangered dipterans, hymenopterans, or coleopterans would be affected.

Ability to control nontarget insect pests: In all of the studies outlined above, there is no evidence that exposure to the CryIA(b) protein expressed in either maize pollen or extracted from *Bt* maize leaves resulted in any toxic effect on the organism tested. Tested organisms include representative avian, aquatic invertebrates, and soil invertebrate species, and several nontarget insect species. Testing with insects known to be either susceptible or not susceptible to native CryIA(b) gave no indications of a changed host specificity for the maize expressed CryIA(b). A small field monitoring study on beneficial insects did not detect any effects on beneficial insect or insect prey species exposed to *Bt* maize. These results suggest that only lepidopterans susceptible to native CryIA(b) are likely to be affected by *Bt* maize *CryIA(b)*.

APHIS concludes that Event 176 Corn exhibit no significant potential to adversely impact organisms beneficial to plants or agriculture or to adversely impact the ability to control nontarget insect pests of agriculture.

F. Cultivation of Event 176 Corn should not reduce the ability to control insects in corn and other crops.

APHIS considered potential impacts associated with the cultivation of Event 176 Corn on the current agricultural practices used to control insects. Ciba Seeds also provided APHIS a copy of their strategy for maximizing the utility of these plants and delaying the development of insect-resistant to the *Btk* insect control protein. The development of effective resistance management strategies is an ongoing process already submitted by the petitioner for EPA consideration, and APHIS will continue to offer comments and recommendations for technical improvements to the EPA and Ciba Seeds to assist in this process. The EPA has stated that they are committed to working with Ciba Seeds to develop product labels and informational brochures that include instructions on the proper use of the Event 176 Corn that are consistent with resistance management.

Both chemical and microbial insecticides are currently used for control of ECB in corn. Among chemical insecticides organophosphates (Counter®, Dyfonate®, Lorsban®, Thimet®, Parathion®, Penncap®), pyrethroids (Ambush®, Pounce®, Capture®), carbamate (Furadan®) and others (Asana XL®) are used. Thougho organophosphates and pyrethroids can be effective against ECB, careful insect surveillance is required. Applications must be carefully timed to reach insect populations before the insects bore into the stalk, and repeated applications are often necessary.

B. thuringiensis var. *kurstaki* preparations are registered for use on corn, vegetables, cotton, deciduous nuts and fruits. As crystalline powder formulations, Btk has been used commercially as an insecticide under the trade name Dipel. Only 5% of the corn is treated with Btk preparations to control ECB. ECB reduces corn yields by causing physical damage to the plant and ear that result in weakness of the stalk dropped ears, and damaged grain. Yield reductions due to ECB infestation are estimated to exceed $50 million annually in the State of Illinois alone. Btk is very effective in the laboratory against ECB. However, commercial *Btk* formulations are generally ineffective in controlling ECB on corn because topical applications of the powder do not reach the inside the corn stalks where the insects feed. Ciba has engineered the Event 176 Corn to produce the Btk insecticide specifically in corn tissue where ECB feeds. The use of this corn should provide farmers a means of controlling a serious insect pest that is not easily controlled by current chemical pesticides. Other advantages include: (1) reducing the risks associated with environmental spills or misapplication of chemical insecticides; (2) eliminating unwanted effects on beneficial insect populations (which can be susceptible to conventional chemical applications); these beneficial insects can, in turn, further reduce the reliance upon chemical means of pest control; and (3) reducing the consumption of fossil fuels required to deliver chemical inputs by machinery.

Event 176 Corn could provide a more effective alternative for season-long control of ECB compared to the use of foliar microbial *Btk* products that nationwide currently only receive limited use. Ciba Seeds field data indicate that Event 176 Corn are more effective than some *Btk* formulations at reducing insect survival and egg-laying. The Event 176 Corn alternative is particularly important where chemical insecticides are no longer effective due to insect resistance. Over the course of use of Event 176 Corn, resistant insects will probably evolve but to recommend a resistance management strategy is outside the scope of this analysis.

Ciba Seeds transgenic Event 176 Corn offers an alternative control method. Ciba Seeds strategies for maximizing the utility of these plants while delaying the development of insect resistance to these plants include the following:

1. Promoting the incorporation of Event 176 Corn into integrated pest management programs (IPM) that emphasize the use of cultural control practices, such as those described above, and judicious and selective use of additional insecticides only when pest populations reach the threshold for economic damage. They do not encourage the use of Event 176 Corn as a stand-alone control measure.

2. Monitoring insect populations for *Btk* protein susceptibility so that development of resistance can be detected and management strategies altered accordingly.

3. High dose expression of *Btk* protein to control insect that are heterozygous for resistance alleles.

4. Deployment of other corn lines or other hosts as refugia for insects that are sensitive to the *Btk* insect control protein, in order to maintain susceptible alleles in the population.

5. Development of new insect control proteins with a distinct mode of action to be employed with the *Btk* protein.

6. Implementation of a grower education program to achieve items 1, 2, and 4 above.

APHIS evaluated the potential impact to agricultural practices associated with the use of Event 176 Corn according to Ciba Seeds strategy. As a result of Ciba Seeds program to instruct growers on the use of cultural control practices and IPM, growers may be more likely to adopt these methods. However, growers will also need to be informed about the implementation of preferred refugia strategies and how these can be integrated with other cultural practices. Growers will be less likely to use chemical insecticides targeted at insect control, and this should reduce the risks associated with some of these insecticides. The use of Event 176 Corn should increase safety to field workers and consumers, reduce toxicity to nontarget species, and lower rates of ground water contamination by insecticides. Event 176 Corn plants are not likely to eliminate completely the use of chemical insecticides, particularly when they may be needed to control other serious pests. But perhaps they may encourage more selective use of insecticides against these pests.

Ciba Seeds support of research in resistance development to Event 176 Corn and their grower education plan, coupled with ECB population monitoring programs, could enable them to implement strategies to delay resistance and detect and possibly contain it when it occurs. It may be possible to control resistant ECB populations by the use of agronomic practices such as crop rotation and alternate insecticides.

APHIS concludes that development of resistance to insecticides is a potential associated with their use; but in this respect, cultivation of Event 176 Corn should pose no greater effects on the control of insects in corn and other crops, than the widely practiced method of applying insecticides.

V. CONCLUSION

APHIS has determined that Event 176 Corn that have previously been field tested under permit, will no longer be considered regulated articles under APHIS 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 those Event 176 Corn or their progeny. (Importation of Event 176 Corn seeds capable of propagation is still, however, subject to the restrictions found in the Foreign Quarantine Notice regulations at 7 CFR Part 319.) This determination has been made based on data collected from these trials, laboratory analyses and literature references presented herein which demonstrate the following:

1. Event 176 Corn exhibits no plant pathogenic properties. Although pathogenic organisms were used in their development, these corn plants are not infected by these organisms nor can these plants incite disease in other plants.

2. Event 176 Corn is no more likely to become a weed than insect-resistant 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 insects would enable corn to become weed pests.

3. Multiple barriers insure that gene introgression from Event 176 Corn into wild or cultivated sexually-compatible plants is extremely unlikely, and such rare events should not increase the weediness potential of any resulting progeny or adversely impact biodiversity.

4. Seeds of Event 176 Corn are substantially equivalent in composition, quality and other characteristics to nontransgenic corn and should have no adverse impacts on raw or processed agricultural commodities.

5. Event 176 Corn exhibits no significant potential to either harm organisms beneficial to the agricultural ecosystem or to impair the ability of farmers to control nontarget insect pests.

6. Cultivation of Event 176 Corn should not reduce the ability to control insects in corn and other crops.

APHIS has also concluded that there will be new varieties bred from Event 176 Corn; however, if such varieties were developed they are unlikely to exhibit new plant pest properties, i.e., properties substantially different from any observed for Event 176 Corn already field tested, or those observed for corn developed from traditional breeding.

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