[Docket No. 95-059-2]

Dekalb Genetics Corporation; Availability of Determination of Nonregulated Status for Corn Line Genetically Engineered for Glufosinate Herbicide Tolerance

AGENCY: Animal and Plant Health Inspection Service, USDA.

ACTION: Notice.

SUMMARY: We are advising the public of our determination that a corn line developed by the Dekalb Genetics Corporation designated as B16 that has been genetically engineered for tolerance to the herbicide glufosinate 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 the Dekalb Genetics Corporation 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 Dekalb Genetics Corporation'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: December 19, 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. Keith Reding, 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 May 25, 1995, the Animal and Plant Health Inspection Service (APHIS) received a petition (APHIS Petition No. 95-145-01p) from the Dekalb Genetics Corporation (Dekalb) of Mystic, CT, seeking a determination that a corn line designated as B16 that has been genetically engineered for tolerance to the herbicide glufosinate does not present a plant pest risk and, therefore, is not a regulated article under APHIS' regulations in 7 CFR part 340.

On August 1, 1995, APHIS published a notice in the Federal Register (60 FR 39146-39147, Docket No. 95-059-1) announcing that the Dekalb 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 the subject corn line posed a plant pest risk. The comments were to have been received by APHIS on or before October 2, 1995.

APHIS received a total of six comments on the subject petition from universities, State departments of agriculture, and an agency of the U.S. government. None of the commenters expressed opposition to the subject petition.

Analysis

Corn line B16 has been genetically engineered with a modified version of the *bar* gene from *Streptomyces hygroscopicus* that encodes a phosphinothricin acetyltransferase (PAT) enzyme. When introduced into the plant cell, the PAT enzyme can inactivate glufosinate herbicides. The *bar* gene was introduced into the subject corn line by microprojectile bombardment, and its expression is under the control of the 35S promoter derived from the plant pathogen cauliflower mosaic virus and the Tr7 terminator from *Agrobacterium tumefaciens*.

Corn line B16 has been considered a regulated article under APHIS' regulations in 7 CFR part 340 because it contains regulatory gene sequences derived from the plant pathogens mentioned above. However, evaluation of field data reports from field tests of the subject corn line conducted under APHIS permits or notifications since 1991 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 Dekalb and a review of other scientific data, comments received, and field tests of the subject corn line, APHIS has determined that corn line B16: (1) Exhibits no plant pathogenic properties; (2) is no more likely to become a weed than corn 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 harm other organisms, including agriculturally beneficial organisms and threatened and endangered species; and (5) should not cause damage to raw or processed agricultural commodities. Therefore, APHIS has concluded that corn line B16 and any progeny derived from hybrid crosses with other nontransformed corn varieties will be just as safe to grow as traditionally bred corn lines that are not regulated under 7 CFR part 340.

The effect of this determination is that a corn line designated as B16 is no

longer considered a regulated article under APHIS' regulations in 7 CFR part 340. Therefore, the notification requirements pertaining to regulated articles under those regulations no longer apply to the field testing, importation, or interstate movement of corn line B16 or its progeny. However, the importation of the subject corn line 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; 60 FR 6000–6005, February 1, 1995). Based on that EA, APHIS has reached a finding of no significant impact (FONSI) with regard to its determination that corn line B16 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 17th day of January 1996.

Terry L. Medley,

Administrator, Animal and Plant Health Inspection Service.

[FR Doc. 96-872 Filed 1-22-96; 8:45 am]

BILLING CODE 3410-34-P

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

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 genetically engineered corn called glufosinate resistant corn transformation event B16. APHIS received a petition from Dekalb Genetics Corporation regarding the status of line B16 as a regulated article under APHIS regulations at 7 CFR Part 340. APHIS has conducted an extensive review of the petition and supporting documentation, as well as 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 glufosinate resistant corn line B16 shall no longer be a regulated article.

John H. Payne, Ph.D.
Acting Director
Biotechnology, Biologics, and Environmental Protection
Animal and Plant Health Inspection Service
U.S. Department of Agriculture
Date: DEC 19 1995

I. SUMMARY

The Animal and Plant Health Inspection Service (APHIS) of the United States Department of Agriculture (USDA), has prepared an Environmental Assessment (EA) before deciding on the regulated status of glufosinate resistant corn line B16 (hereafter referred to as line B16). The developer of line B16, the Dekalb Genetics Corporation, petitioned APHIS requesting a determination on the status of line B16 and any progeny derived from it as regulated articles under APHIS regulations found at 7 CFR Part 340 (hereafter referred to as the regulations). The petition contained information pertinent to the company's contention that line B16 does not present a plant pest risk and therefore, should no longer be a regulated article under the APHIS regulations. Line B16 has been considered a regulated article because it was engineered with DNA sequences derived from the plant pathogens, cauliflower mosaic virus (CaMV)and *Agrobacterium tumefaciens*. As a regulated article, APHIS approval has been required for introductions (importation, interstate movements, and field tests) of line B16.

Line B16 was developed by using recombinant DNA techniques to introduce a modified version of a *bar* gene, which encodes the enzyme phosphinothricin acetyl transferase (PAT). PAT can detoxify glufosinate-ammonium (GA) herbicides and thereby confer resistance or tolerance. The *pat* gene was originally isolated from the common soil microorganism, *Streptomyces hygroscopicus*. After isolation, the *bar* gene was modified to enable efficient expression of the gene in plants. The modified *bar* gene then was engineered into a line of corn via particle bombardment.

GA is in the phosphinothricin class of herbicides. It is a non-systemic, non-selective herbicide used for post-emergence control of many broadleaf and grassy weeds. GA kills plants by inhibiting the enzyme glutamine synthetase (GS), the only enzyme in plants that can detoxify the ammonia generated by various metabolic processes within the plant.

EAs were prepared by APHIS before granting the permits for field trials with line B16. Previous EAs addressed questions pertinent to plant pest risk issues for conducting field trials under physical and reproductive confinement, but they did not address several issues that are relevant to the unconfined growth of line B16. With respect to these new issues, APHIS concludes that the transformed line B16:

1. exhibits no plant pathogenic properties. Although DNA sequences from a plant pathogen were used in their development, these corn plants are not infected nor can these plants incite disease in other plants;

2. is no more likely to become a weed than corn developed by traditional breeding techniques. Corn is not considered to be a serious, principal or common weed pest in the U.S.;

3. is unlikely to increase the weediness potential for any other cultivated or wild species with which it can interbreed. The introgression of the bar gene from line B16 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. will not harm other organisms, including agriculturally beneficial organisms and threatened and endangered species; and,

5. should not cause damage to processed agricultural commodities. Seeds of lineB16 are not significantly different in composition, quality, and other characteristics to the nontransgenic parental corn and should have no adverse impacts on raw or processed agricultural commodities.

Therefore, after a review of the available evidence, APHIS believes that line B16 will be just as safe to grow as traditionally-bred corn varieties that are not subject to APHIS regulation under 7 CFR Part 340. APHIS concludes that there will be no significant impact on the human environment if line B16 or its progeny are no longer considered regulated articles under the regulations.

II. BACKGROUND

Development of line B16.
In a petition dated May 24, 1995, Dekalb Genetics Corporation requested a determination from APHIS that glufosinate resistant corn line B16, and any progeny derived from them, should no longer be considered a regulated article under APHIS regulations found at 7 CFR Part 340. The glufosinate resistant corn line B16 has been considered a regulated article because it was engineered with DNA sequences derived from the plant pathogens cauliflower mosaic virus (CaMV) and *Agrobacterium tumefaciens*.

Line B16 was developed by using recombinant DNA techniques to introduce a modified version of a *bar* gene, which encodes the enzyme phosphinothricin acetyltransferase, PAT. PAT can detoxify glufosinate-ammonium herbicides and thereby confer resistance or tolerance. The *bar* gene was originally isolated from the common soil microorganism, *Streptomyces hygroscopicus*. The modified *bar* gene then was engineered into a parental corn line using particle bombardment.

GA is in the phosphinothricin class of herbicides. It is a non-systemic, non-selective herbicide used for post-emergence control of many broadleaf and grassy weeds. GA kills plants by inhibiting the enzyme glutamine synthetase, the only enzyme in plants that can detoxify the ammonia generated by various metabolic processes within the plant.

Line B16 has been field tested since 1991 in the major corn growing regions of the United States under permits and acknowledgements of notifications from APHIS (USDA Permit Numbers 90-332-02, 90-332-04, 91-317-01, 92-034-01, 92-365-04, 93-014-02; and Notification Numbers 94-081-02, 94-081-04, 94-081-05, 94-088-01, 94-109-06). The corn line has also been field tested in Argentina and Canada. Line B16 has been evaluated extensively in laboratory, greenhouse, and field experiments to confirm that they exhibit the desired agronomic characteristics and do not pose a plant pest risk. Although the field tests of line B16 has 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.

Section 340.6 of the regulations, entitled "Petition Process for Determination of Nonregulated Status", provides that a person may petition APHIS to evaluate submitted information 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, APHIS 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. Line B16 is also subject to regulation by other agencies. APHIS' decision on the regulatory status of line B16 under APHIS' regulations at 7 CFR 340, does not release this corn and its progeny from EPA or FDA regulatory oversight. 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.*). Therefore, any use of herbicides on line B16 will be regulated by EPA. 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 line B16 as a regulated article under APHIS regulations. The developer of line B16, Dekalb Genetics Corporation, submitted a petition to APHIS requesting that APHIS make a determination that line B16 and their progeny shall no longer be considered regulated articles under APHIS regulations (7 CFR Part 340).

This EA was prepared in compliance 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; 60 FR 6000-6005, February 1, 1995).

IV. ALTERNATIVES

A. No Action.

Under the Federal "no action" alternative, APHIS would not come to a determination that line B16 is no longer a regulated article under the regulations at 7 CFR Part 340. Permits from APHIS would still be required for introductions of line B16. APHIS might choose this alternative if there were insufficient evidence to demonstrate the lack of plant pest risk from uncontained cultivation of line B16.

B. Determination that line B16 is no longer a regulated article.

Under this alternative, line B16 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 line B16. A basis for this determination would include a "Finding of No Significant Impact" under NEPA.

V. AFFECTED ENVIRONMENT AND POTENTIAL ENVIRONMENTAL IMPACTS

This EA addresses potential environmental impacts from an APHIS determination that line B16 should no longer be considered a regulated article. Previous EAs prepared by APHIS with the issuance of permits for field tests of line B16 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 line B16.

Additional technical information is included in the determination document appended to this EA, and is incorporated by reference. The determination includes more detailed discussions of the biology of corn, the genetic components used in the construction of line B16, and the analyses that lead APHIS to conclude that line B16 have no potential to pose a plant pest risk.

A. Potential for the introduced genes, their products, and the added regulatory sequences controlling their expression to present a plant pest risk in LINE B16

Line B16 was developed by introducing a synthetic version of the *bar* gene derived from the common microorganism *S.* hygroscopicus. The *bar* gene encodes the enzyme PAT which inactivates GA and related herbicides. After isolation from *S. hygroscopicus*, the *bar* gene was modified to enable the gene to be expressed in plants. The resultant PAT enzyme is indistinguishable from the PAT produced in *S. hygroscopicus*. Although part of the modification of the bar gene included adding sequences from a plant virus (CaMV), line B16 is not infected nor does it pose a plant pest risk. The sequences from CaMV are well characterized and widely used to facilitate expression of genes engineered into plants via recombinant DNA techniques. Once inserted into the chromosome of the corn plant, the introduced bar gene is maintained and transmitted in the same manner as any other genes.

B. Potential for line B16 to become a successful weed

Corn has been grown for centuries throughout the world without any reports that it is a serious weed pest, and it is unlikely to become a 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 line B16 is a line that exhibits no appreciable weedy characteristics. The bar gene is unlikely to increase weediness of line B16. The glufosinate resistance of these plants will confer a selective advantage only when glufosinate is applied to the plants. No other attributes of line B16 suggest that it be any more "weedy" than traditionally-bred corn cultivars. Other than the resistance to the herbicide glufosinate, line B16 has retained the agronomic

characteristics of the parental corn, including the sensitivity to other herbicides.

Dekalb Genetics Corporation has provided data regarding agronomic performance including yield characteristics, disease and pest susceptibilities, compositional analyses, and numerous other tests which support APHIS' conclusion that line B16 is no more likely to become a weed than corn developed by traditional breeding techniques.

C. Potential for line B16 to increase the weediness potential of any other plant with which it can interbreed.

Cultivated corn and the wild, related species of *Zea* can be crossed to produce fertile offspring. However in nature, such 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).

APHIS considered whether the movement of the *bar* gene from line B16 to other cultivated corn or wild relatives might result in offspring that would present problems as weeds. The genetic integrity of commercial cultivated corn lines and varieties is carefully controlled through established plant breeding practices. These standard practices make it unlikely that this glufosinate resistance trait will be inadvertently incorporated into the germplasm of cultivated corn lines.

D. Potential for line B16 to harm other organisms, including agriculturally beneficial organisms and threatened or endangered species.

Consistent with its statutory authority and requirements under NEPA, APHIS evaluated the potential for line B16 to directly or indirectly harm other organisms, including those that are recognized as beneficial to agriculture and those that are recognized as threatened or endangered in the United States.

APHIS concluded that the available evidence suggests that line B16 will not have a significant adverse impact on organisms beneficial to plants or agriculture, nontarget organisms, and will not harm threatened or endangered species.

The use of GA and related herbicides in the cultivation of line B16 or their offspring will be regulated by the EPA under its existing regulations for the registration of pesticide use. As part of the pesticide registration process, EPA considers the impacts on the environment, including organisms.

E. Potential for line B16 to damage agricultural commodities.

APHIS can envision no way in which line B16 would damage agricultural commodities. With the exception of the single enzyme, PAT, the composition and attributes of line B16 are indistinguishable from the parental line of corn used to develop it. There is no indication that the PAT enzyme itself will affect the qualities of commodities derived from line B16.

VI. CONCLUSION

APHIS has evaluated information from the scientific literature as well as information submitted by Dekalb Genetics Corporation that characterized line B16. After careful analysis, APHIS has identified no significant impact to the environment from issuance of a determination that line B16 should no longer be a regulated article under APHIS regulations at 7 CFR Part 340. This finding is supported by the following conclusions that line B16:

1. exhibits no plant pathogenic properties. Although DNA sequences from a plant pathogen were used in their development, these corn plants are not infected nor can these plants incite disease in other plants;

2. is no more likely to become a weed than corn developed by traditional breeding techniques. Corn is not considered to be a serious, principal or common weed pest in the U.S.;

3. is unlikely to increase the weediness potential for any other cultivated or wild species with which it can interbreed. The introgression of the *bar* gene from line B16 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. will not harm other organisms, including agriculturally beneficial organisms and threatened and endangered species;

5. should not cause damage to processed agricultural commodities. Seeds of line B16 are not significantly in composition, quality, and other characteristics to nontransgenic parental variety and should have no adverse impacts on raw or processed agricultural commodities.

Therefore, after review of the available evidence, APHIS concludes that line B16 will be just as safe to grow as traditionally-bred 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 line B16 were no longer considered a regulated article under its regulations at 7 CFR Part 340.

VII. LITERATURE CITED

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

Galinat, W. C. 1988. The Origin of Corn. In: Sprague, G. F., Dudley, J. W., Editors. Corn and Corn Improvement, Third Edition. pp. 1-31. American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America, Madison, Wisconsin. 986 pp.

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.

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

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Response to the Dekalb Genetics Corporation Petition for a Determination of Nonregulated Status for Glufosinate Resistant Corn Line B16

Prepared by
United States Department of Agriculture
Animal and Plant Health Inspection Service
Biotechnology, Biologics, and Environmental Protection

I. SUMMARY

In a petition dated May 24, 1995, Dekalb Genetics Corporation requested a determination from the Animal and Plant Health Inspection Service (APHIS) that glufosinate resistant corn line B16, and any progeny derived from it, should no longer be considered a regulated article under APHIS regulations found at 7 CFR Part 340. The glufosinate resistant corn line B16 has been considered a regulated article because it was engineered with DNA sequences derived from the plant pathogens, cauliflower mosaic virus (CaMV) and *Agrobacterium tumefaciens*.

Line B16 was developed by using recombinant DNA techniques to introduce a modified version of a *bar* gene, which encodes the enzyme phosphinothricin acetyltransferase, PAT. PAT can detoxify glufosinate-ammonium herbicides and thereby confer resistance or tolerance. The *bar* gene was originally isolated from the common soil microorganism *Streptomyces hygroscopicus*. After isolation, the *bar* gene was modified by (1) attaching noncoding DNA regulatory sequences from CaMV and (2) altering codon usage of the *bar* coding region to enhance expression of the *bar* gene in plants (the resultant amino acid sequence of PAT was not altered). The modified *bar* gene then was engineered into a parental corn line using particle bombardment.

Glufosinate-ammonium (GA) is in the phosphinothricin class of herbicides. It is a non-systemic, non-selective herbicide used for post-emergence control of many broadleaf and grassy weeds. GA kills plants by inhibiting the enzyme glutamine synthetase (GS), the only enzyme in plants that can detoxify the ammonia generated by various metabolic processes within the plant (e.g., photorespiration, nitrate reduction, and amino acid degradation). The inhibition of GS leads to the accumulation of phytotoxic levels of ammonia in the plant.

Based on a review of available scientific information, APHIS has determined that line B16 does not present a plant pest risk and therefore is no longer a regulated article under the regulations found at 7 CFR Part 340. Because of this determination, oversight under these regulations will no longer be required from APHIS for field testing, importation, or interstate movement of line B16 or its progeny.

This determination has been made based on an analysis that revealed that line B16: (1) exhibits no plant pathogenic properties; (2) is no more likely to become a weed than herbicide-tolerant corn lines developed by traditional breeding techniques; (3) is unlikely to increase the weediness potential of any other cultivated plant or native wild species with which the organisms can interbreed; (4) will not harm other organisms, such as bees, which are beneficial to agriculture; and 5) does not cause damage to processed agricultural commodities. APHIS has also concluded that there is no reason to believe that new progeny corn varieties derived from line B16 will exhibit new plant pest properties, i.e., properties substantially different from any observed for the B16 corn line already field tested, or those observed for corn in traditional breeding programs.

II. BACKGROUND

APHIS Regulatory Authority. APHIS regulations found at 7 CFR Part 340 (hereafter referred to as the regulations) 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. The regulations pertain to the introduction (importation, interstate movement, or release into the environment) of certain genetically engineered organisms and products.

A genetically engineered organism is considered a regulated article if the donor organism, recipient organism, vector, or vector agent used in engineering the organism belongs to one of the taxa listed in the regulation and is also a plant pest, or if there is reason to believe that it is a plant pest. Line B16 has been considered a "regulated article" under Part 340 of the regulations because they have been engineered with certain noncoding regulatory sequences derived from the plant pathogenic virus, cauliflower mosaic virus (CaMV).

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 information 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. As a consequence of such a determination, APHIS permits would no longer be required for field testing, importation, or interstate movement of that article or its progeny.

APHIS' decision on the regulatory status of line B16 under APHIS' regulations at 7 CFR 340, does not release this corn and its progeny from EPA and FDA regulatory oversight. The regulation of herbicide use, including the use of glufosinate on corn, is under the jurisdiction of the EPA.

III. RESPONSE TO COMMENTS

APHIS received a total of six comments on the Dekalb petition from universities, state departments of agricultue, and an agency of the U.S. Federal government. None of the commenters expressed opposition to the subject petition.

IV. ANALYSIS OF Line B16

Biology of Corn (Maize). Cultivated corn or maize (*Zea mays*) is a member of the family Gramineae (grass family). The genus *Zea* contains four species, but only *Z. mays* has been developed so dramatically from the other members of the genus and from its wild ancestors. Because of concerted human intervention over centuries of selection and plant breeding, corn bears little resemblance to its relatives. Much of the agronomic development of corn has focussed on the production of large, nutritious seeds (kernels) that do not shatter from the plant upon maturity. The kernels remain tight within the ear, allowing maximum grain harvest and minimal dissemination of the seed.

Researchers believe that the domestication of *Z. mays* was centered in a region of Mexico near Mexico City (Galiant, 1988). By the time of Columbus' expedition to the Americas, corn development and production had spread from Chile to Canada. It was Columbus who brought corn to Europe where it spread within two generations to virtually all regions of the world where corn growth was possible.

Corn is now grown worldwide and used primarily for animal feed, human food, and for the production of materials used in industry. According to agricultural statistics for 1993 and 1994, (USDA, 1994), approximately 22% of the world's total corn seed is planted in the United States, yielding 45% of the world production. In the United States corn exceeds all other major crops with regard to acres harvested and crop value.

Rationale for Development of Glufosinate Resistant Corn. Several herbicides are currently available for weed management in corn. Weed management is a critical factor for corn yield, and growers typically favor herbicide management strategies that control a broad spectrum of weed species, will not injure the crop, are cost effective, and have positive environmental attributes. Several classes of herbicides have effective broad spectrum weed control if used either singly or in combination; however, they may injure or kill some crops when used at the application rates suggested for weed control.

GA, the active ingredient in the herbicides Basta®, Ignite®, Liberty®, Finale®, Rely® and , is an amino acid analogue which exhibits broad spectrum, non-systemic, and non-selective herbicidal activity (Leason et al., 1982; Weld and Wendler, 1990). GA herbicides are used for post-emergence control of many broadleaf and grassy weeds. GA kills plants by inhibiting the enzyme glutamine synthetase (GS), the only enzyme in plants that can detoxify the ammonia generated by various metabolic processes within the plant. GA exhibits low residual activity, low soil leaching, and low toxicity to nontarget organisms. GA is readily degraded by microorganisms in the soil.

For years, pre-emergence herbicides have been the major tool used for weed control in conventional corn production. Pre-emergence herbicide treatments are applied prior to, or at the time of planting, before the crop and weed seedlings emerge from the soil.

With the development of effective post-emergence herbicides and increased use of no-tillage corn, growers frequently seek to control weeds when and where they emerge. Depending on the incidence, timing, and density of weed species in a crop field, the grower can use post-emergence herbicides only as necessary to achieve the desired level of weed control. The use of GA as an effective post emergence herbicide with glufosinate resistant corn may make it possible to reduce the use of pre-emergence herbicides in corn production.

Development of line B16
Line B16 contains a stably integrated *bar* gene which encodes the enzyme PAT. PAT catalyzes the conversion of L-phosphinothricin, the active ingredient in GA, to an inactive form and thereby confers resistance to the herbicide. The *bar* gene used to develop line B16 is a slightly modified synthetic version of the original *pat* gene isolated from *S.* hygroscopicus (see details below).

Parent Embryonic Cells from Hybrid A188 x B73. An embryonic cell suspension culture from line A188 x B73 was used for the transformation. This genotype was selected based on the ability to establish and maintain callus and suspension clutlure that are capable of regeneration of fertile plants (Kamo and Hodges, 1986). A188 is a non-commercial inbred that was recognized for its amenability in tissue culture. B73 is a stiff stalk inbred that has been used extensively in commercial corn hybrids. The strategy for development glufosinate resistant corn is to use traditional backcrossing and breeding to produce commercial hybrids with a wide range of genotypes.

The *bar* Gene Used to Develop line B16. Line B16 contains a synthetic version of the *pat* gene derived from the soil microorganism *S. hygroscopicus*, (ATCC 21705). The *bar* gene of *S. hygroscopicus* is similar to the *S.* viridochromogenes *pat* gene which also encodes a phosphinothricin acetyltransferase (Hara et al., 1991). These PAT enzymes are believed to be part of a defense mechanism of some strains of streptomycetes which produce a class of antibiotic compounds (bialaphos, phosphinothricin) and a PAT enzyme to protect itself from the inhibitory effects of the antibiotic (Kumada et al., 1988).

To conform with plant codon usage, the GTG initiation codon present in the *S. hygroscopicus* gene was mutated to ATG. The PAT protein, which is comprised of 183 amino acids with a molecular weight of about 21,000, is not altered.

Construction of the Plasmid Used for Transformation. The plasmid pDPG165 was used to transform the parental tissue culture hybrid line A188 x B73. This plasmid was derived by inserting the bar expression cassette into the high copy number *Escherichia coli* plasmid pUC19 (Yanisch-Perron et al., 1985). This construct contains the *bar* gene (White et al., 1990) with the associated *35S* gene promoter from cauliflower mosaic virus (Odell et al., 1985) and Tr7 terminator derived from *Agrobacterium tumefaciens* (Dhaese et al., 1983). In addition, the plasmid contains an ampicillin resistance (*bla*) gene (Sutcliffe, 1978) from plasmid pBR322 and a bacterial origin of replication. The *bla* gene has regulatory sequences recognized in bacterium but are not functional in the transgenic corn cells. Therefore, the *bar* gene is the only introduced gene that can be expressed in the plant cells.

Protoplast Transformation System. Plasmid DNA was introduced into embryonic maize by microprojectile bombardment (Gordon-Kamm et al., 1990). A suspension of DNA coated micorprojectile particles was loaded onto a macrooprojectile, which was accelerated by a gunpowder blast. Particles penetrated the target maize cell, and DNA was released from the particles. The DNA then integrated into a chromosome of a maize cell. The cells were returned to a liquid medium and cultured for 1-2 weeks in the absence of selective pressure. The putatively transformed cells were cultivated on plates containing 3 mg/L bialaphos. Transformants were identified by the presence of phosphinothrin acetyltransferase enzyme activity and Southern blot analysis, demonstrating the presence of the *bar* gene.

A. THE INTRODUCED GENES, THEIR PRODUCTS, AND THE ADDED REGULATORY SEQUENCES CONTROLLING THEIR EXPRESSION DO NOT PRESENT A PLANT PEST RISK IN LINE B16.

As summarized above, the genetic construct was introduced via microprojectile bombardment. Southern blot analyses indicate that line B16 contain a single copy of the pDPG165 plasmid. Once inserted into the chromosome of the corn plant, the introduced genes are maintained and transmitted in the same manner as any other genes.

Expression of the *bar* gene in line B16 is modulated by noncoding DNA regulatory sequences derived from the plant pathogen, CaMV. Specifically, these regulatory sequences are the CaMV promoter (Odell et al., 1985) and Tr7 *Agrobacterium tumefaciens* terminator (Dhaese et al., 1983). These regulatory sequences are utilized widely in the expression of genes engineered into plants. Although these regulatory sequences are derived from a plant pathogen, there is no evidence to suggest that they pose a plant pest risk in this corn line.

B. LINE B16 HAS NO SIGNIFICANT POTENTIAL TO BECOME A SUCCESSFUL WEED.

Corn is not considered a weed. Many of the changes involved in the domestication of corn from teosinte and wild type maize have resulted in a domesticated corn plant that exhibits high yielding capacity, non-shattering of mature seed and ease in harvest, but these changes also have led to a species unable to exist on its own in the wild. Also lost was a perennial nature and the ability of domestic maize seed to remain viable in the soil for long periods. The many agronomic traits that make maize an outstanding crop species also make it largely dependent on humans for its survival. In the United States, corn that is grown in rotation with soybeans may volunteer on occasion. However, this volunteer corn can be readily controlled with an array of commercial graminicides registered for use in soybeans.

A weed pest is a plant that grows persistently in locations where it is unwanted. Corn has been grown for centuries throughout the world without any reports that it is a serious weed pest. In the United States, it is not a species listed under the Federal Noxious Weed Act. Corn is not classified as a serious, principal, or common weed pest (Holm et al., 1979). Corn is considered a highly domesticated, well-characterized crop plant that is not persistent in undisturbed environments without human intervention.

Evaluations of line B16 in laboratory, greenhouse, and field tests support the conclusion that line B16 has little potential to become a weed pest. Volunteers of line B16 can be controlled using plysical methods or with the use of other herbicides. With the exception of the resistance to GA, line B16 has agronomic traits similar to those of traditionally bred corn and does not exhibit traits that cause concern that they might become a weed pest.

C. LINE B16 WILL NOT INCREASE THE WEEDINESS POTENTIAL OF ANY OTHER PLANT WITH WHICH IT CAN INTERBREED.

APHIS considered whether the movement of the *bar* gene from line B16 to other cultivated corn or wild relatives might result in offspring that would present problems as weeds. The genetic integrity of commercial cultivated corn lines and varieties is carefully controlled through established plant breeding practices. These standard practices make it unlikely that this glufosinate resistance trait will be

inadvertently incorporated into the germplasm of cultivated corn lines.

APHIS also considered the likelihood of introgression of the *bar* gene into non-cultivated or wild species that are related to corn. In the case of corn, pollination of its nearest relatives with corn pollen is extremely unlikely to yield fertile offspring. The scientific literature indicates that it is unlikely that there will be any significant introgression of genes from corn into non-cultivated relatives (Doebley, 1984).

D. LINE B16 WILL NOT HARM ORGANISMS BENEFICIAL TO AGRICULTURE OR ORGANISMS THAT ARE DESIGNATED AS THREATENED OR ENDANGERED.

APHIS evaluated the potential for line B16 plants to harm organisms either directly or indirectly, particularly those organisms that are recognized as beneficial to agriculture. There is no reason to believe that the cultivation of line B16 corn or their progeny will exert any deleterious effects on organisms recognized as beneficial to agriculture. Likewise, cultivation of line B16 will not harm any species designated as threatened or endangered. Line B16 produces a single enzyme, PAT, that is not produced in nontransgenic corn. There is no indication that this enzyme is toxic to beneficial organisms or results in the production of toxic constituents. In addition, APHIS can envision no plausible mechanism whereby line B16 would be injurious or pathogenic to beneficial organisms such as bees and earthworms.

The definition of line B16 encompasses not only the corn lines that already have been field tested, but also new corn lines produced through conventional breeding using line B16 as one or both parents. APHIS believes that the analysis applied to the line B16 plants already field tested will apply equally well to these new corn lines, and that the data provided by Dekalb Genetics Corporation justify the conclusion that such new lines derived from line B16 will not present a plant pest risk. The variation in agronomic characteristics among the line B16 plants that have been field tested does not differ significantly from that seen in commercial cultivars of corn that have never been considered regulated articles. Therefore, there is no reason to believe that any of the progeny of line B16 will possess plant pest properties.

E. LINE B16 SHOULD NOT CAUSE DAMAGE TO PROCESSED AGRICULTURAL COMMODITIES.

The characteristics of line B16 corn have no known attributes that could have an indirect plant pest effect on any processed plant commodity. During extensive testing in the laboratory, greenhouse and in the field, plants of line B16 exhibited agronomic characteristics typical of the parent corn. In APHIS' opinion, the components and processing characteristics of line B16 reveal no differences in any component that could have an indirect plant pest effect on any processed plant commodity.

IV. CONCLUSION

APHIS has determined that corn line B16, which has been field tested previously under permit, will no longer be considered a regulated article under APHIS regulations found at 7 CFR Part 340. Permits under those regulations will no longer be required from APHIS for field testing, importation, or interstate movement of those corn or their progeny. However, the importation of line B16 corn and vegetative plant material or seeds capable of propagation are still subject to the restrictions found in foreign quarantine notices in 7 CFR Part 319.

This determination has been made based on information from field trials, laboratory analyses, and literature references presented herein which demonstrate that line B16:

1) exhibits no plant pathogenic properties;

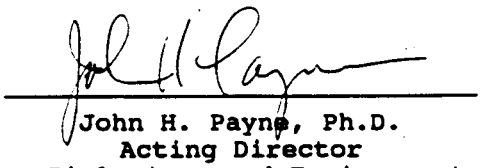
2) is no more likely to become a weed than herbicide-tolerant corn developed by traditional breeding techniques;

3) is unlikely to increase the weediness potential for any other cultivated or wild species with which they can interbreed;

4) will not harm other organisms, including agriculturally beneficial organisms and threatened and endangered species; and

5) should not cause damage to processed agricultural commodities.

APHIS has also concluded that there is a reasonable certainty that new progeny of line B16 or varieties bred from these lines will not exhibit new plant pest properties, i.e., properties substantially different from any observed for line B16 plants already field tested, or those observed for corn in traditional breeding programs.



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Date: DEC 19 1995

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