Notices

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This section of the FEDERAL REGISTER contains documents other than rules or proposed rules that are applicable to the public. Notices of hearings and investigations, committee meetings, agency decisions and rulings, delegations of authority, filing of petitions and applications and agency statements of organization and functions are examples of documents appearing in this section.

DEPARTMENT OF AGRICULTURE

Animal and Plant Health Inspection Service

[Docket No. 95-011-2]

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 corn developed by AgrEvo USA Company designated as Glufosinate Resistant Corn Transformation Events T14 and T25 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 AgrEvo USA Company in its petition for a determination of nonregulated status, an analysis of other scientific data, and our review of comments received from the public in response to a previous notice announcing our receipt of the AgrEvo USA Company 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: June 22, 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. David Heron, 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 December 23, 1994, the Animal and Plant Health Inspection Service (APHIS) received a petition (APHIS Petition No. 94-357-01p) from AgrEvo USA Company (AgrEvo) of Wilmington, DE, seeking a determination that corn designated as Glufosinate Resistant Corn (GRC) Transformation Events T14 and T25 (GRC Events T14 and T25) 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 February 27, 1995, APHIS published a notice in the **Federal Register** (60 FR 10537-10538, Docket No. 95-011-1) announcing that the AgrEvo petition had been received and was available for public review. The notice also discussed the role of APHIS, the Environmental Protection Agency, and the Food and Drug Administration in regulating the subject corn 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 28, 1995.

APHIS received nine comments on the AgrEvo petition. Comments were received from associations, universities, seed companies, and a State department of agriculture. All the commenters supported the AgrEvo petition for nonregulated status for the subject corn.

Analysis

GRC Events T14 and T25 contain a gene that encodes the enzyme phosphinothricin-N-acetyltransferase (PAT). The PAT enzyme catalyzes the conversion of L-phosphinothricin, the active ingredient in glufosinate-ammonium, to an inactive form, thereby conferring resistance to herbicides in the phosphinothricin class. The *pat* gene in GRC Events T14 and T25 is a synthetic version of the gene isolated from the bacterium *Streptomyces viridochromogenes*. Expression of the *pat* gene is regulated by the 35S promoter and the 35S terminator derived from the plant pathogen cauliflower mosaic virus. 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 a plant pathogen. However, evaluation of field data reports from field tests of the subject corn conducted under APHIS permits or notifications since 1992 indicate 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 AgrEvo and a review of other scientific data, comments received from the public, and field tests of the subject corn, APHIS has determined that GRC Events T14 and T25: (1) Exhibit no plant pathogenic properties; (2) are no more likely to become weeds than other corn developed by traditional breeding techniques; (3) are unlikely to increase the weediness potential for any other cultivated or wild species with which they can interbreed; (4) will not harm other organisms, such as bees, that are beneficial to agriculture; and (5) should 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 GRC Events T14 and T25 will exhibit new plant pest properties, i.e., properties substantially different from any observed for the GRC Events T14 and T25 already field tested or those observed for corn in traditional breeding programs.

The effect of this determination is that corn designated as GRC Events T14 and T25 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 GRC Events T14 and T25 or their 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 GRC Events T14 and T25 and lines developed from them 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 6th day of July 1995.

Terry L. Medley,

Acting Administrator, Animal and Plant Health Inspection Service.

[FR Doc. 95–17079 Filed 7–13–95; 8:45 am]

BILLING CODE 3410–34–P

USDA/APHIS Petition 94-357-01 for Determination of Nonregulated Status for Glufosinate Resistant Corn Transformation Events T14 and T25

Environmental Assessment and
Finding of No Significant Impact

June 1995

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 events T14 and T25. APHIS received a petition from AgrEvo USA Company regarding the status of transformation events T14 and T25 as regulated articles 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 transformation events T14 and T25 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: JUN 22 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 transformation events T14 and T25 (hereafter referred to as T14 and T25). The developer of T14 and T25, the AgrEvo USA Company, petitioned APHIS requesting a determination on the status of T14 and T25, and any progeny derived from them, 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 T14 and T25 do not present a plant pest risk and therefore, should no longer be regulated articles under the APHIS regulations. T14 and T25 have been considered regulated articles because they were engineered with DNA sequences derived from the plant pathogen, cauliflower mosaic virus (CaMV). As regulated articles, APHIS approval has been required for introductions (importation, interstate movements, and field tests) of T14 and T25.

T14 and T25 were developed by using recombinant DNA techniques to introduce a modified version of a *pat* 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 viridochromogenes*. After isolation, the *pat* gene was modified to enable efficient expression of the gene in plants. The modified *pat* gene then was engineered into a line of yellow dent corn via direct uptake of DNA by corn protoplasts.

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 T14 and T25. Previous EAs addressed questions pertinent to plant pest risk issues relevant to the conduct of field trials under physical and reproductive confinement, but they did not address several issues that are relevant to the unconfined growth of T14 and T25. With respect to these new issues, APHIS concludes the following:

1. T14 and T25 exhibit 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. T14 and T25 are no more likely to become weeds 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. T14 and T25 are unlikely to increase the weediness potential for any other cultivated or wild species with which they can interbreed. The introgression of the *pat* gene from T14 and T25 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. T14 and T25 will not harm other organisms, including agriculturally beneficial organisms and threatened and endangered species.

5. T14 and T25 should not cause damage to processed agricultural commodities. Seeds of T14 and T25 are substantially equivalent in composition, quality, and other characteristics to nontransgenic yellow dent corn and should have no adverse impacts on raw or processed agricultural commodities.

Therefore, after a review of the available evidence, APHIS believes that T14 and T25 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 T14 and T25 or their progeny were no longer considered regulated articles under the regulations.

II. BACKGROUND

Development of T14 and T25. In a petition dated December 23, 1994, AgrEvo USA Company requested a determination from APHIS that T14 and T25, and any progeny derived from them, should no longer be considered regulated articles under APHIS regulations found at 7 CFR Part 340. T14 and T25 have been considered regulated articles because they were engineered with DNA sequences derived from the plant pathogen, CaMV.

T14 and T25 were developed by using recombinant DNA techniques to introduce a modified version of a *pat* gene, which encodes the enzyme PAT. PAT can detoxify glufosinate-ammonium herbicides and thereby confer resistance or tolerance. The *pat* gene was originally isolated from the common soil microorganism, *S. viridochromogenes*. After isolation, the *pat* gene was modified to enable efficient expression of the gene in plants. The modified *pat* gene then was engineered into a line of yellow dent corn via direct uptake of DNA by corn protoplasts.

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.

T14 and T25 have been field tested since 1992 in the major corn growing regions of the United States under permits and acknowledgements of notifications from APHIS (USDA Permit Numbers 92-017-04, 92-043-01, 93-021-10, 93-021-11; and Notification Numbers 93-120-17, 93-120-27, 94-074-03)). T14 and T25 have 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 T14 and T25 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.

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. T14 and T25 are also subject to regulation by other agencies. APHIS' decision on the regulatory status of T14 and T25 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 T14 and T25 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 T14 and T25 as a regulated article under APHIS regulations. The developer of T14 and T25, AgrEvo USA Company, submitted a petition to APHIS requesting that APHIS make a determination that T14 and T25 and their progeny shall no longer be considered regulated articles under APHIS regulations (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 T14 and T25 are no longer regulated articles under the regulations at 7 CFR Part 340. Permits from APHIS would still be required for introductions of T14 and T25. APHIS might choose this alternative if there were insufficient evidence to demonstrate the lack of plant pest risk from uncontained cultivation of T14 and T25.

B. Determination that T14 and T25 is no longer a regulated article.

Under this alternative, T14 and T25 would no longer be regulated articles under the regulations at 7 CFR Part 340. Permits from APHIS would no longer be required for introductions of T14 and T25. 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 T14 and T25 should no longer be considered regulated articles. Previous EAs prepared by APHIS with the issuance of permits for field tests of T14 and T25 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 T14 and T25.

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 T14 and T25,

and the analyses that lead APHIS to conclude that T14 and T25 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 transformation events T14 and T25

T14 and T25 were developed by introducing a synthetic version of the *pat* gene derived from the common microorganism *S. viridochromogenes*. The *pat* gene encodes the enzyme PAT which inactivates GA and related herbicides. After isolation from *S. viridochromogenes*, the *pat* gene was modified to enable the gene to be expressed in plants. The resultant PAT enzyme is indistinguishable from the PAT produced in *S. viridochromogenes*. Although part of the modification of the *pat* gene included adding sequences from a plant virus (CaMV), T14 and T25 are not infected nor do they 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 *pat* gene is maintained and transmitted in the same manner as any other genes.

B. Potential for T14 and T25 to become successful weeds

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 T14 and T25 is a line of yellow dent corn that exhibits no appreciable weedy characteristics. The *pat* gene is unlikely to increase weediness of T14 and T25. The glufosinate resistance of these plants will confer a selective advantage only when glufosinate is applied to the plants. No other attributes of T14 and T25 suggest that it be any more "weedy" than traditionally-bred corn cultivars. Other than the resistance to the herbicide glufosinate, T14 and T25 have retained the agronomic characteristics of the parental corn, including the sensitivity to other herbicides.

AgrEvo USA Company has provided data regarding seed germination rates, yield characteristics, disease and pest susceptibilities, compositional analyses, and numerous other tests which support APHIS' conclusion that T14 and T25 are no more likely to become weeds than corn developed by traditional breeding techniques.

C. Potential for T14 and T25 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 *pat* gene from T14 and T25 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 assiduously 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 T14 and T25 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 T14 and T25 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 T14 and T25 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 T14, T25, 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 T14 and T25 to damage agricultural commodities.

APHIS can envision no way in which T14 and T25 would damage agricultural commodities. With the exception of the single enzyme, PAT, the composition and attributes of T14 and T25 are indistinguishable from the parental line of corn used to develop T14 and T25. There is no indication that the PAT enzyme itself will affect the qualities of commodities derived from T14 and T25.

VI. CONCLUSION

APHIS has evaluated information from the scientific literature as well as information submitted by AgrEvo USA Company that characterized T14 and T25. After careful analysis, APHIS has identified no significant impact to the environment from issuance of a determination that T14 and T25 should no longer be regulated articles under APHIS regulations at 7 CFR Part 340. This finding is supported by the following conclusions:

1. T14 and T25 exhibit 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. T14 and T25 are no more likely to become weeds 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. T14 and T25 are unlikely to increase the weediness potential for any other cultivated or wild species with which they can interbreed. The introgression of the *pat* gene from T14 and T25 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. T14 and T25 will not harm other organisms, including agriculturally beneficial organisms and threatened and endangered species.

5. T14 and T25 should not cause damage to processed agricultural commodities. Seeds of T14 and T25 are substantially equivalent in composition, quality, and other characteristics to nontransgenic yellow dent corn and should have no adverse impacts on raw or processed agricultural commodities.

Therefore, after review of the available evidence, APHIS concludes that T14 and T25 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 T14 and T25 were no longer considered regulated articles 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.

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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 AgrEvo USA Company's Petition for a Determination of Nonregulated Status for Glufosinate Resistant Corn Transformation Events T14 and T25

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

I. SUMMARY

In a petition dated December 23, 1994, AgrEvo USA Company requested a determination from the Animal and Plant Health Inspection Service (APHIS) that glufosinate resistant corn transformation events T14 and T25, and any progeny derived from them, should no longer be considered regulated articles under APHIS regulations found at 7 CFR Part 340. The glufosinate resistant corn transformational events T14 and T25 (hereafter called T14 and T25) have been considered regulated articles because they were engineered with DNA sequences derived from the plant pathogen, cauliflower mosaic virus (CaMV).

T14 and T25 were developed by using recombinant DNA techniques to introduce a modified version of a *pat* gene, which encodes the enzyme PAT. PAT can detoxify glufosinate-ammonium herbicides and thereby confer resistance or tolerance. The *pat* gene was originally isolated from the common soil microorganism, *Streptomyces viridochromogenes*. After isolation, the *pat* gene was modified by (1) attaching noncoding DNA regulatory sequences from CaMV and (2) altering codon usage of the *pat* coding region to enhance expression of the *pat* gene in plants (the resultant amino acid sequence of PAT was not altered). The modified *pat* gene then was engineered into a line of yellow dent corn via direct uptake of DNA by corn protoplasts.

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 T14 and T25 do not present a plant pest risk and therefore are no longer regulated articles 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 T14 and T25 or their progeny.

This determination has been made based on an analysis that revealed that T14 and T25: (1) exhibit no plant pathogenic properties; (2) are no more likely to become weeds than corn lines developed by traditional breeding techniques; (3) are 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) do 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 T14 and T25 will exhibit new plant pest properties, i.e., properties substantially different from any

observed for the T14 and T25 corn lines 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. T14 and T25 have been considered "regulated articles" 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 T14 and T25 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 is under the jurisdiction of the EPA.

III. RESPONSE TO COMMENTS

APHIS received a total of nine comments on the petition from the following: a state department of agriculture (1); seed companies (2); growers' associations (3) and universities (3). All comments were favorable to the substance of the petition.

IV. ANALYSIS OF T14 AND T25

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®, Rely® and Finale™, 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 has the advantage of 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 advent of effective post-emergence herbicides and an 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 only as much herbicide 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 T14 and T25

T14 and T25 are yellow dent corn material that contain a stably integrated *pat* 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 *pat* gene used to develop T14 and T25 is a synthetic version of the *pat* gene isolated from *S. viridochromogenes* (see details below).

Parent Tissue Culture Line He/89. Tissue culture line He/89 was used for the transformation. It was developed at AgrEvo GmbH (formerly Hoechst Ag, Frankfurt, Germany) from parents developed at the Cereal Breeding Institute in Szeged, Hungary. Primary transformation events T14 and T25 were selected for commercial development. They have been crossed with both commercially available public inbred lines and proprietary inbred lines of the yellow dent type. The commercialization strategy for glufosinate resistant corn is to use traditional backcrossing and breeding to transfer the glufosinate resistance locus from T14 and T25 to a wide range of varieties with a wide range of maturities.

The *pat* Gene Used to Develop T14 and T25. T14 and T25 contain a synthetic version of the *pat* gene derived from *S. viridochromogenes*, strain Tü 494 (Bayer et al., 1972). The *pat* gene of *S. viridochromogenes* is similar to the *S. hygroscopicus bar* (bialaphos resistance) 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).

Because the native *pat* gene (i.e., from *S. viridochromogenes*) has a high G:C content that is atypical for plants, a modified nucleotide sequence was synthesized using codons preferred by plants. The amino acid sequence of the PAT enzyme remains unchanged, and transformed plants exhibit resistance to GA (Eckes et al., 1989).

Construction of the Plasmid Used for Transformation. The plasmid p35S/AC was used to transform the parental tissue culture line He/89. This plasmid is based upon the pUC-derived plasmid pDH51 (Pietrzak et

al., 1986) and contains the synthetic *pat* gene with the associated *35S* gene promoter and terminator sequences derived from CaMV. In addition, the plasmid contains an ampicillin resistance (*ampR*) gene and a bacterial origin of replication. The *ampR* gene has regulatory sequences recognized in bacteria but not functional in the transgenic corn cells. Therefore, the *pat* gene is the only introduced gene which can be expressed in the plant cells.

Protoplast Transformation System. Researchers at AgrEvo GmbH introduced the plasmid DNA into corn protoplasts by a direct uptake technique. Protoplasts and DNA were mixed together in a buffered solution while a polyethylene glycol solution was added dropwise. After gentle mixing and incubation at room temperature the protoplasts were gently pelleted, washed and resuspended in a protoplast culture medium. The putatively transformed protoplasts were cultivated under various conditions until microcolonies of more than 20-50 plant cells were formed. The microcolonies then were transferred to solid medium and grown for several weeks before selection of transformants on medium containing L-PPT, which has the same activity as GA. Fertile corn plants were regenerated from corn protoplasts as described by Mórocz and colleagues (1990).

A. THE INTRODUCED GENES, THEIR PRODUCTS, AND THE ADDED REGULATORY SEQUENCES CONTROLLING THEIR EXPRESSION DO NOT PRESENT A PLANT PEST RISK IN T14 AND T25.

As summarized above, the genetic construct was introduced via direct uptake of plasmid DNA by corn protoplasts. Southern blot and polymerase chain reaction (PCR) analyses indicate that T14 and T25 contain 3 and 1 copies of the *pat* gene, respectively. Once inserted into the chromosome of the corn plant, the introduced *pat* gene is maintained and transmitted in the same manner as any other genes.

Expression of the *pat* gene in T14 and T25 is modulated by noncoding DNA regulatory sequences derived from the plant pathogen, CaMV. Specifically, these regulatory sequences are the promoter and 3'-nontranslated regions of the CaMV *35S* transcript (Pietrzak, 1986). 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.

B. T14 AND T25 HAVE NO SIGNIFICANT POTENTIAL TO BECOME SUCCESSFUL WEEDS.

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 domestic 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 T14 and T25 in laboratory, greenhouse, and field tests support the conclusion that T14 and T25 have little potential to become weed pests. With the exception of the resistance to GA, T14 and T25 have agronomic traits similar to those of traditionally bred corn and do not exhibit traits that cause concern that they might become weed pests.

C. T14 AND T25 WILL NOT INCREASE THE WEEDINESS POTENTIAL OF ANY OTHER PLANT WITH WHICH IT CAN INTERBREED.

APHIS considered whether the movement of the *pat* gene from T14 and T25 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 *pat* 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. Based upon a review of the scientific literature, APHIS concludes that it is unlikely that there will be any significant introgression of genes from corn into non-cultivated relatives (Doebley, 1984).

D. T14 AND T25 WILL NOT HARM ORGANISMS BENEFICIAL TO AGRICULTURE OR ORGANISMS THAT ARE DESIGNATED AS THREATENED OR ENDANGERED.

APHIS evaluated the potential for T14 and T25 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 T14 and T25 corn or their progeny will exert any deleterious effects on organisms recognized as beneficial to agriculture. Likewise, cultivation of T14 and T25 will not harm any

species designated as threatened or endangered. T14 and T25 produce 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 T14 and T25 would be injurious or pathogenic to beneficial organisms such as bees and earthworms.

The definition of T14 and T25 encompasses not only the corn lines that already have been field tested, but also new corn lines produced through conventional breeding using T14 and T25 as one or both parents. APHIS believes that the analysis applied to the T14 and T25 plants already field tested will apply equally well to these new corn lines, and that the data provided by AgrEvo USA Company justify the conclusion that such new lines derived from T14 and T25 will not present a plant pest risk. The variation in agronomic characteristics among the T14 and T25 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 T14 and T25 will possess plant pest properties.

E. T14 AND T25 SHOULD NOT CAUSE DAMAGE TO PROCESSED AGRICULTURAL COMMODITIES.

The characteristics of T14 and T25 corn have no apparent 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 T14 and T25 exhibited the typical agronomic characteristics of the parent corn. In APHIS' opinion, the components and processing characteristics of T14 and T25 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 T14 and T25 that previously have been field tested under permit will no longer be considered regulated articles 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 T14 and T25 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:

1) T14 and T25 exhibit no plant pathogenic properties;

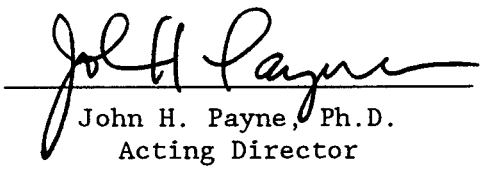
2) T14 and T25 are no more likely to become weeds than corn developed by traditional breeding techniques;

3) T14 and T25 are unlikely to increase the weediness potential for any other cultivated or wild species with which they can interbreed;

4) T14 and T25 will not harm other organisms, including agriculturally beneficial organisms and threatened and endangered species; and

5) T14 and T25 should not cause damage to processed agricultural commodities.

APHIS has also concluded that there is a reasonable certainty that new progeny of T14 and T25 or varieties bred from these lines will not exhibit new plant pest properties, i.e., properties substantially different from any observed for T14 and T25 plants already field tested, or those observed for corn in traditional breeding programs.



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