[Docket No. 95-076-2]

Plant Genetic Systems (America), Inc.; Availability of Determination of Nonregulated Status for Corn Line Genetically Engineered for Male Sterility and Glufosinate Herbicide Tolerance as a Marker

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 Plant Genetic Systems (America), Inc., designated as event MS3 that has been genetically engineered for male sterility and tolerance to the herbicide glufosinate as a marker 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 Plant Genetic Systems (America), Inc., 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 Plant Genetic Systems (America), Inc., 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: February 22, 1996.

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. James White, 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; E-mail: mkpeterson@aphis.usda.gov.

SUPPLEMENTARY INFORMATION:

Background

On August 16, 1995, the Animal and Plant Health Inspection Service (APHIS) received a petition (APHIS Petition No. 95-228-01p) from Plant Genetics Systems (America), Inc., (PGS) of Des Moines, IA, seeking a determination that a corn line designated as transformation MS3 (event MS3) that has been genetically engineered for male sterility and tolerance to the herbicide glufosinate as a marker does not present a plant pest risk and, therefore, is not a regulated article under APHIS' regulations in 7 CFR part 340.

On November 16, 1995, APHIS published a notice in the Federal Register (60 FR 57570-57571, Docket No. 95-076-1) announcing that the PGS 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 January 16, 1996.

APHIS received a total of six comments on the subject petition from seed companies, State departments of agriculture, and a seed farm. All of the comments were in support of the subject petition.

Analysis

Event MS3 has been genetically engineered with a gene from *Bacillus amyloliquefaciens* encoding a ribonuclease called barnase, which inhibits pollen formation and results in male sterility of the transformed plants. The subject corn line also contains the *bar* gene isolated from the bacterium *Streptomyces hygroscopicus* that encodes a phosphinothricin acetyltransferase (PAT) enzyme, which, when introduced into a plant cell, inactivates glufosinate. Linkage of the *barnase* gene, which induces male sterility, with the bar gene, a glufosinate tolerance gene used as a marker, enables identification of the male sterile line

before the plant begins to flower. Event MS3 was transformed via immature embryo electroporation in yellow dent corn material. Expression of the introduced genes is controlled in part by the P35S promoter derived from the plant pathogen cauliflower mosaic virus and the 3'nos sequence from the plant pathogen *Agrobacterium tumefaciens*.

Event MS3 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 1992 indicates that there were no deleterious effects on plants, nontarget organisms, or the environment as a result of the subject corn plants' release into the environment.

Determination

Based on its analysis of the data submitted by PGS and a review of other scientific data, comments received, and field tests of the subject corn line, APHIS has determined that corn line event MS3: (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 threatened or endangered species or other organisms, such as bees, which are beneficial to agriculture; and (5) will not cause damage to raw or processed agricultural commodities. Therefore, APHIS has concluded that corn line event MS3 and any progeny derived from hybrid crosses with other nontransformed corn varieties will not exhibit new plant pest properties, i.e., properties substantially different from any observed for event MS3 corn plants already field tested, or those observed for corn in traditional breeding programs.

The effect of this determination is that PGS' corn line designated as event MS3 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 PGS' corn line event MS3 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 event MS3 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 29th day of February, 1996.

Terry L. Medley,

Acting Administrator, Animal and Plant Health Inspection Service.

[FR Doc. 96–5376 Filed 3–6–96; 8:45 am]

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USDA/APHIS Petition 95-228-01 for Determination of Nonregulated Status for MS3 Corn

Environmental Assessment and
Finding of No Significant Impact

February 1996

The Animal and Plant Health Inspection Service (APHIS) of the U. S. Department of Agriculture has conducted an environmental assessment before issuing a determination of nonregulated status for a genetically engineered corn called MS3 Corn. APHIS received a petition from the Plant Genetic Systems (America) Inc. regarding the status of the MS3 Corn as a regulated article under APHIS regulations at 7 CFR Part 340. APHIS has conducted an extensive review of the petition, supporting documentation, and other relevant scientific information. Based upon the analysis documented in this environmental assessment, APHIS has reached a finding of no significant impact on the environment for its determination that male sterile MS3 Corn 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: FEB 12 1996

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I. SUMMARY

The Animal and Plant Health Inspection Service (APHIS), United States Department of Agriculture (USDA), has prepared an Environmental Assessment (EA) before deciding on the regulated status of a genetically engineered line of male sterile corn designated hereafter as MS3 Corn. The developer of MS3 Corn, the Plant Genetic Systems (America) Inc. petitioned APHIS requesting a determination on the regulated status of MS3 Corn that has been a regulated article under APHIS regulations. Interstate movements and field tests of MS3 Corn have been conducted under permits issued by or notifications acknowledged by APHIS. Plant Genetic Systems has petitioned APHIS for a determination that MS3 Corn does not present a plant pest risk and should, therefore, no longer be a regulated article under APHIS regulations at 7 CFR Part 340.

The MS3 Corn has been developed to provide a more reliable system to generate hybrid seed corn by genetically engineering a plant to be male sterile. To generate a male sterile corn plant, a ribonuclease called barnase, has been engineered into corn. The ribonuclease, which is expressed only in the tapetum cells of the pollen sac during anther development, blocks pollen development thus, resulting in a male sterile plant. Expression of barnase in these cells apparently results in degradation of host RNAs, thus blocking pollen cell development. A selectable genetic marker encoding phosphinothricin acetyltransferase has also been introduced into the corn chromosome to facilitate selection of transformed cells in the laboratory and identification of male fertile plants during plant breeding. The genes were introduced via a well-characterized procedure that results in direct introduction of genes into plant genomes.

EA's have been previously prepared before granting the permits for MS3 Corn field trials. Previous EA's addressed questions pertinent to plant pest risk issues concerning the conduct of field trials under physical and reproductive confinement, but the EA's did not address several issues that are of relevance to the unconfined growth of MS3 Corn. With respect to these new issues, APHIS concludes the following:

1. MS3 Corn exhibits no plant pathogenic properties. Although components of pathogenic organisms were used in their development, these corn plants are not infected by these organisms nor can these plants incite disease in other plants.

2. MS3 Corn is no more likely to become a weed than male sterile corn that has been developed by traditional breeding techniques. Corn is not a serious, principal, or common weed pest in the U.S. and there is no reason to believe that male sterility would enable corn to become a weed pest.

3. Multiple barriers, including sterility of this line, insure that gene introgression from MS3 Corn into wild or cultivated sexually-

compatible plants is extremely unlikely, and such rare events should not increase the weediness potential of any resulting progeny or adversely impact biodiversity.

4. Seeds of MS3 Corn are substantially equivalent in composition, quality and other characteristics to nontransgenic corn and should have no adverse impacts on raw or processed agricultural commodities.

5. MS3 Corn exhibits no significant potential to either harm threatened or endangered species or organisms beneficial to the agricultural ecosystem.

Therefore, after a review of the available evidence, APHIS believes that MS3 Corn will be just as safe to grow as traditionally bred male sterile corn varieties not subject to regulation under 7 CFR Part 340. APHIS concludes that there will be no significant impact on the human environment if MS3 Corn is no longer considered a regulated article under regulations at 7 CFR Part 340.

II. BACKGROUND

Plant Genetic Systems has submitted a "Petition for Determination of Non-regulated Status" for corn plants that contain a gene that blocks pollen development, thereby producing a male sterile plant. Plant Genetic Systems requested a determination from APHIS that the MS3 Corn, and any progeny derived from hybrid crosses between this line and other non-transformed corn varieties, no longer be considered regulated articles under 7 CFR Part 340.

Development of MS3 Corn. Virtually all the corn grown in the U.S. for food and feed is hybrid corn. Hybrid corn, unlike their inbred parents, are vigorous, uniform, and productive due to heterosis. A number of hybrid corn seed production systems are used to ensure hybridization by forced pollination between the female and male parental corn lines. Artificial emasculation (detassling) and male sterility genetic-based systems are currently the most poplar. Detasseling involves the physical removal of the tassel from the female plant, either manually or in combination with mechanical devices. The detasseling is probably the most difficult to manage of any of the steps in hybrid corn seed production (Wych, 1988) because the time period is so short. Manual detasseling is labor intensive and expensive. About 100,000 people nationwide may be needed for as little as a week but not more than five weeks.

Male sterile plants can be produced genetically via traditional breeding. One type of sterility is encoded in the nucleus while the other is cytoplasmically inherited. One problem with these sterility genes is that they lack a selectable marker that would allow their identification in a hybrid seed breeding program prior to flowering. If some of the female fertile plants are male-fertile, their pollen cannot be eliminated prior to flowering. This pollen could compete

with the pollen from the intended male fertile plants and result in seed that is not the intended hybrid seed.

To generate a male sterile corn plant, a ribonuclease called barnase, was engineered into corn. The ribonuclease, which is expressed only in the tapetum cells of the pollen sac during anther development, blocks pollen development resulting in a male sterile plant. Expression of barnase in these cells apparently results in degradation of host RNAs blocking cell development. A selectable genetic marker encoding phosphinothricin acetyltransferase from *Streptomyces hygroscopicus* was introduced into the corn chromosome to facilitate selection of transformed cells in the laboratory and identification of male fertile plants during plant breeding. The genes were introduced via a well-characterized procedure that results in direct introduction of genes into plant genomes. Thus, the advantage of MS3 Corn is that its male-sterility can be verified, by the expression of the marker gene phosphinothricin acetyltransferase, prior to flowering.

MS3 Corn has been field tested by PGS and its partners in the major corn growing regions of the United States under permits and acknowledgements of notifications since 1992. MS3 Corn has been evaluated extensively in laboratory, greenhouse, and field experiments to confirm that it exhibits the desired agronomic characteristics and does not pose a plant pest risk. Although the field tests of MS3 Corn have been conducted in agricultural settings, the permit conditions and acknowledgement of notifications for the tests have stipulated physical and reproductive confinement.

APHIS Regulatory Authority. APHIS regulations at 7 CFR Part 340, which were promulgated pursuant to authority under 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, affect 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 regulations and is also a plant pest, or there is reason to believe that it is a plant pest. MS3 Corn described in the Plant Genetic Systems petition has been considered a regulated article because noncoding DNA regulatory sequences are derived from plant pathogens.

Section 340.6 of the regulations, entitled "Petition Process for Determination of Nonregulated Status", provides that a person may petition the Agency to evaluate submitted data and determine that a particular regulated article does not present a plant pest risk and should no longer be regulated. If APHIS determines that the regulated article is unlikely to pose a greater plant pest risk than the unmodified organism, the Agency can grant the petition in whole or in part. Thereafter, 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. MS3 Corn may also be subject to regulation by other agencies. The EPA is responsible for the regulation of pesticides under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136 *et seq.*). FIFRA requires that all pesticides, including herbicides, be registered before distribution or sale, unless exempt by EPA regulation. In cases in which the genetically modified plants allow for a new use of an herbicide or involve a different use pattern for the herbicide, the EPA must approve the new or different use. In making such an approval, the EPA considers the possibility of adverse effects to human health and the environment from the use of this herbicide. When the use of the herbicide on the genetically modified plant would result in an increase in the residues of the herbicide in a food or feed crop for which the herbicide is currently registered, or in new residues in a crop for which the herbicide is not currently registered, establishment of a new tolerance or a revision of the existing tolerance would be required. Residue tolerances for pesticides are established by the EPA under the Federal Food, Drug and Cosmetic Act (FFDCA) (21 U.S.C. 201 *et seq.*). The Food and Drug Administration (FDA) enforces tolerances set by the EPA under the FFDCA.

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 MS3 Corn as a regulated article under APHIS regulations. The developer of MS3 Corn, Plant Genetic Systems (America) Inc., has submitted a petition to USDA, APHIS requesting that APHIS make a determination that MS3 Corn shall no longer be considered a regulated article under 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 MS3 Corn is no longer a regulated article under the regulations at 7 CFR Part 340. Permits from APHIS would still be required for introductions of MS3 Corn. APHIS might choose this alternative if there were insufficient evidence to demonstrate the lack of plant pest risk from uncontained cultivation of MS3 Corn.

B. Determination that MS3 Corn is no longer a regulated article.

Under this alternative, MS3 Corn would no longer be a regulated article under the regulations at 7 CFR Part 340. Permits from APHIS would no longer be required for introductions of MS3 Corn. A basis for this determination would include a "Finding of No Significant Impact" under the National Environmental Policy Act of 1969 (42 USC 4321 *et seq.*; 40 CFR 1500-1508; 7 CFR Part 1b; 7 CFR Part 372.

V. AFFECTED ENVIRONMENT AND POTENTIAL ENVIRONMENTAL IMPACTS

This EA addresses potential environmental impacts from a determination that MS3 Corn should no longer be considered a regulated article under APHIS regulations at 7 CFR Part 340. Previous EA's prepared by APHIS with the issuance of permits for field tests of MS3 Corn have addressed various attributes of this corn. This EA discusses the genetic modification, and the potential environmental impacts that might be associated with the unconfined cultivation of MS3 Corn.

Additional technical information is included in the determination document appended to this EA, and incorporated by reference. This includes detailed discussions of the biology of corn, the genetic components used in the construction of MS3 Corn, and the analyses that lead APHIS to conclude that MS3 Corn has no potential to pose plant pest risks.

A. Potential impacts based on increased weediness of MS3 Corn relative to other male sterile corn

Although various definitions of the term "weed" have been proposed in the scientific literature, the salient point is that a plant can be considered a weed when it is growing where humans do not want it (Baker 1965; de Wet and Harlan 1975; Muenscher 1980). Baker (1965) lists 12 common attributes that can be used to assess the likelihood that a plant species will behave as a weed. Keeler (1989) and Tiedje et al. (1989) have adapted and analyzed Baker's list to develop admittedly imperfect guides to the weediness potential of transgenic plants. Both authors emphasize the importance of looking at the parent plant and the nature of the specific genetic changes.

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

Furthermore, corn has been grown for centuries throughout the world without any reports that it is a serious weed pest. In the United States, corn is not listed as a weed in the major weed references (Crockett 1977; Holm et al. 1979; Muenscher 1980), nor is it listed as a noxious weed species by the Federal Government (7 CFR Part 360).

The parent plant of the MS3 Corn is an agricultural crop plant that exhibits no appreciable weedy characteristics. The relevant introduced trait, male sterility, is unlikely to increase weediness of MS3 Corn. There is no indication that the presence of a specific ribonuclease, called barnase, will convert corn into a weed. The corn plants have also been transformed with a gene phosphinothricin acetyltransferase, which confers resistance to the herbicide glufosinate. The gene has no involvement in plant disease or damage. Also, its use does not result in the presence of the herbicide in corn and does not indicate that glufosinate will be used in the cultivation of the corn. No other attributes of MS3 Corn suggest that it be any more "weedy" than the present corn cultivars that are the result of traditional breeding. The MS3 Corn has retained the agronomic characteristics of the parental corn. Plant Genetic Systems has provided data regarding seed germination rates, yield characteristics, disease and pest susceptibilities, compositional analyses, and numerous other test reported in the Plant Genetic Systems' application that support APHIS' conclusion that MS3 Corn is just as safe to grow as any other male sterile corn.

B. Potential impacts on the sexually-compatible relatives of corn arising from pollination by MS3 Corn

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

The closest relative to *Zea* is *Tripsacum*, a genus of seven species, three of which occur in the United States (Gould 1968). *Tripsacum* differs from corn in many respects, including chromosome number (n=9), in contrast to *Zea* (n=10). All species of *Tripsacum* can cross with *Zea*, but only with difficulty and resulting seeds are sterile (Galinat 1988).

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

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

Our analysis of the biology of cultivated male sterile corn and its relatives leads us to predict that the environmental impacts of cultivation of MS3 Corn anywhere in the world would be no different from impacts attributable to similar varieties produced with traditional breeding techniques. The species *Z.mays* is native to Mexico and Central America. Non-cultivated varieties of *Zea* sp. have coexisted and co-evolved in the Americas over millennia. Even if MS3 Corn were to be cultivated in agricultural regions around centers of *Zea* diversity, there is no reason to expect impacts from MS3 Corn to be significantly different from those arising from the cultivation of any other variety of male sterile corn.

International traffic in MS3 Corn would be fully subject to national and regional phytosanitary standards promulgated under the International Plant Protection Convention (IPPC). The IPPC has set a standard for the reciprocal acceptance of phytosanitary certification among the nations that have signed or acceded to the Convention (98 countries as of December 1992). The treaty, now administered by a

Secretariat housed within the United Nations Food and Agriculture Organization in Rome, came into force on April 3, 1952. It establishes standards to facilitate the safe movement of plant materials across international boundaries. Plant biotechnology products are fully subject to national legislation and regulations, or regional standards and guidelines promulgated under the IPPC. The vast majority of IPPC signatories have promulgated, and are now administering such legislation or have prepared guidelines. These signatories includes Mexico, which has in place a regulatory process requiring a full evaluation of MS3 Corn before it can be introduced into the environment. The IPPC has also led to the creation of Regional Plant Protection Organizations (RPPOs) such as the North American Plant Protection Organization (NAPPO). Our trading partners will be kept informed of our regulatory decisions through NAPPO and other fora. Our decision in no way prejudices regulatory action in any other country.

All the considerable, existing national and international regulatory authorities and phytosanitary protocols that apply to introductions of new male sterile varieties apply equally to those covered by this analysis.

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

Consistent with its statutory authority and requirements under NEPA, APHIS evaluated the potential for MS3 corn, and plant products derived from it, to have damaging or toxic effects directly or indirectly on nontarget organisms, particularly those that are recognized as beneficial to agriculture and to those that are recognized as threatened or endangered in the United States.

Plant Genetic System's analysis of MS3 Corn identified no toxic components that are present in concentrations significantly different from concentrations in nontransgenic corn. The genetic modification in MS3 corn does not result in the production of new proteins, enzymes, or metabolites in the plant that are known to have toxic properties. The plants also do not exhibit any pathogenic properties.

APHIS concludes that the unconfined growth of MS3 corn, and products derived from it, will have no deleterious effects on organisms recognized as beneficial to agriculture (e.g., earthworms, honey bees) or on other organisms, including any species recognized as threatened or endangered in the United States.

Because MS3 genes would only be in half of the hybrid seeds sold to farmers, use of this male sterility system does not necessarily provide farmers herbicide tolerant corn. However, if the other parent in the hybrid breeding system was phosphinothricin-tolerant, then plants derived from that hybrid seed would be herbicide tolerant and

the farmers could potentially apply the herbicide. The use of phosphinothricin-class of herbicides in the cultivation of MS3 Corn 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 nontarget organisms.

D. **Potential impacts on agricultural and cultivation practices.**

Based on its analysis, APHIS concludes that there is unlikely to be any significant adverse impact on agricultural practices associated with the use of MS3 Corn.

E. **MS3 Corn will not cause damage to processed agricultural commodities.**

In APHIS' opinion, the components and processing characteristics of MS3 Corn reveal no differences in any component that could have an indirect plant pest effect on any processed plant commodity.

VI. CONCLUSION

APHIS has evaluated information from the scientific literature as well as data submitted by Plant Genetic Systems (America) Inc. that characterized MS3 Corn. After careful analysis, APHIS has made a finding of no significant impact to the environment from issuance of a determination that MS3 Corn should no longer be a regulated article under APHIS regulations at 7 CFR Part 340. That finding is supported by the following conclusions:

1. MS3 Corn exhibits no plant pathogenic properties. Although components of pathogenic organisms were used in their development, these corn plants are not infected by these organisms nor can these plants incite disease in other plants.

2. MS3 Corn is no more likely to become a weed than male sterile corn that has been developed by traditional breeding techniques. Corn is not a serious, principal, or common weed pest in the U.S. and there is no reason to believe that male sterility would enable corn to become a weed pest.

3. Multiple barriers, including sterility of this line, insure that gene introgression from MS3 Corn into wild or cultivated sexually-compatible plants is extremely unlikely, and such rare events should not increase the weediness potential of any resulting progeny or adversely impact biodiversity.

4. Seeds of MS3 Corn are substantially equivalent in composition, quality and other characteristics to nontransgenic corn and should

have no adverse impacts on raw or processed agricultural commodities.

5. MS3 Corn exhibits no significant potential to either harm threatened or endangered species or organisms beneficial to the agricultural ecosystem.

Therefore, after review of the available evidence, APHIS concludes that MS3 Corn will be just as safe to grow as traditionally bred, male-sterile 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 MS3 Corn were no longer considered a regulated article under its regulations (7 CFR Part 340).

VII. LITERATURE CITED

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VIII. PREPARERS AND REVIEWERS

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Response to Plant Genetic System's Petition for a Determination of Nonregulated Status for Male Sterile MS3 Corn

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

I. SUMMARY

In a petition dated August 16, 1995, Plant Genetic Systems (America) requested a determination from the Animal and Plant Health Inspection Service (APHIS) that male sterile corn MS3 Corn and any progeny derived from it, should no longer be considered regulated articles under APHIS regulations at 7 CFR Part 340. The male sterile corn transformational event MS 3 (hereafter called MS3 Corn) have been considered regulated articles because they were engineered with DNA sequences derived from the plant pathogens, cauliflower mosaic virus (CaMV) and *Agrobacterium tumefaciens*.

Based on a review of available scientific information, APHIS has determined that MS3 Corn does not present a plant pest risk and therefore is no longer a regulated article under the regulations 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 MS3 Corn or their progeny.

This determination has been made based on an analysis that revealed that MS3 Corn: (1) exhibits no plant pathogenic properties; (2) is no more likely to become a weed than corn lines developed by traditional breeding techniques; (3) is unlikely to increase the weediness potential of any other cultivated plant or native wild species with which MS3 Corn can interbreed; (4) will not harm threatened and endangered species and other organisms, such as bees, which are beneficial to agriculture; and 5) will 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 MS3 Corn will exhibit new plant pest properties, i.e., properties substantially different from any observed for the MS3 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 regulations and is also a plant pest, or there is reason to believe that it is a plant pest. MS3 Corn 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 pathogens, cauliflower mosaic virus (CaMV) and *Agrobacterium tumefaciens*.

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 MS3 Corn 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 6 comments on the petition from the following: a state department of agriculture (2) and seed companies (4). All comments were favorable to the petition.

IV. ANALYSIS OF MS3 Corn

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 Male Sterile Corn. Virtually all the corn grown in the U.S. for food and feed is hybrid corn. Hybrid corn, unlike their inbred parents, is vigorous, uniform and high yielding. A number of hybrid corn seed production systems are in use to ensure hybridization by forced pollination between the female and male parental corn lines. Artificial emasculation (detassling) and male sterility genetic-based systems are currently the most poplar. Detasseling involves the physical removal of the tassel from the female plant, either manually or in combination with mechanical devices. Detasseling is probably the most difficult to manage of any of the steps in hybrid corn seed production (Wych, 1988). The detasseling period is short. Manual detasseling is labor intensive and expensive operation. About 100,000 people may be needed for a little as a week but not more than five weeks every year.

Male sterile plants can be produced genetically via traditional breeding. One type of sterility genes is encoded in nucleus while the other is cytoplasmically inherited. One of the problems of using these sterility genes is that they lack a selectable marker that would allow their identification in a hybrid seed breeding program before flowering. Breeders need a simple way of eliminating the male fertile segregants before flowering to the design of an efficient hybrid seed production scheme. The most common system used today uses a mix of male sterile plants emasculated by detassling mixed with genetically (cytoplasmically) sterile plants. Pollen is provided by a limited number of male fertile plants in the field.

Development of MS3 Corn. To make a male sterile corn plant, a ribonuclease called barnase, has been engineered into corn. The ribonuclease, which is expressed only in the tapetum cells of the pollen sac during anther development, blocks pollen development, thus producing a male sterile plant. To direct the expression of barnase to pollen cells, a pollen specific promoter, PTA 29 (Seurinck et al., 1990) was used to direct the enzyme's synthesis. Expression of barnase in pollen cells apparently results in degradation of host RNAs and blocking development of mature viable pollen. Barnase is an extracellular ribonuclease from *Bacillus amyloliquefaciens*, a common soil microorganism. *B. amyloliquefaciens* also contains an intracellular protein called barstar which specifically inhibits barnase by combining with it in a one-to-one complex (Hartely 1989). Thus, barstar is produced intracellularly by the same organism that secretes barnase (Hartely and Smeaton 1973).

The second gene inserted into the MS3 genome encodes tolerance to a herbicide. Phosphinothricin (PPT) is an analogue of glutamine that inhibits glutamine synthase (GS) of bacteria and plants. Bialaphos is a tripeptide precursor of PPT produced by Streptomyces spp., in which two alanine molecules are linked to the PPT moiety. The active PPT is released by the action of intracellular peptidases. To protect

against the toxic effects of such antibiotics, Streptomyces hygroscopicus produces an acetyltransferase enzyme (abbreviated PAT) that acetylates PPT or its precursor bialophos and inactivates the molecule. Thompson et al. (1987) isolated the PAT gene and DeBlock et al. (1987) showed that expression of the gene in transgenic tobacco plants conferred tolerance to PPT and bialophos. The chemically synthesized PPT is called glufosinate and tradenames of the herbicide are Basta®, Finale®, or Ignite®. The PAT enzyme is produced in MS3 corn under the direction of cauliflower mosaic virus 35S promoter and nopaline synthase termination/polyadenylation sequences. Although MS3 Corn is tolerant to the herbicide, hybrid progeny (the seeds that would be available to the farmers) developed from this line may or may not be tolerant to the herbicide. If the female parent is not herbicide tolerant, then only half of the progeny hybrid corn would carry the tolerance gene. This would mean that farmers could not apply the herbicide because 50 per cent of the plants would die. However, if the female parent in the hybrid selction scheme was glufosinate tolerant, all the progeny would herbicide tolerant and farmers could apply the herbicide (assuming the herbicide is registered by EPA for use on corn).

The above two genes were introduced via a well-characterized procedure that results in direct introduction of genes into plant genomes. In this instance, immature corn embryos were transformed via electroporation. As a result of using this technique several other genes and DNA sequences present on the plasmids were introduced into the genome. A complete copy of β-lactamase from *E. coli*, which encodes ampicillin-carbenicillin resistance, is also present in the MS 3 genome. Plant Genetic System has demonstrated that the gene which was engineered with bacterial-specific promoters is not expressed in plants. Even in the highly unlikely occurrence of this gene becoming expressed by the plant, the antibiotic ampicillin would not be produced by the plant. This gene acts by inactivating the antibiotic. Also, present is a sequence called *ori* (origin of replication), which is required for the replication of the plasmids in *E. coli*. It should have no function in the plant. Also present in the MS 3 genome are partial copies of the barstar gene and chloramphenicol acetyl transferase. As stated above, barstar is the protein which specifically inhibits barnase. Barstar was present on a plasmid in *E. coli* along with barnase and PAT containing plasmid. The presence of barstar was necessary to ensure that barnase was not expressed in *E. coli* that could result in poor cell growth. Chloramphenicol acetyltransferase encodes resistance to the chloramphenicol class of antibiotics. Both of these partial genes do not possess plant-specific promoters and even if expressed would not pose any risk.

A. THE INTRODUCED GENES, THEIR PRODUCTS, AND THE ADDED REGULATORY SEQUENCES CONTROLLING THEIR EXPRESSION DO NOT PRESENT A PLANT PEST RISK IN MS3 CORN.

As summarized above, the genetic construct was introduced via direct uptake of plasmid DNA by corn embryos. Once inserted into the

chromosome of the corn plant, the introduced genes are maintained and transmitted in the same manner as any other genes as demonstrated by Mendelian data presented by applicant.

Expression of the phosphinothricin acetyltransferase in MS3 Corn is directed by noncoding DNA regulatory sequences derived from the plant pathogens cauliflower mosaic virus and *A. tumefaciens*. These regulatory sequences are utilized widely in the expression of genes engineered into plants (Odell et al., 1985; Weising et al., 1988). Although these regulatory sequences are derived from a plant pathogen, there is no evidence to suggest that they pose a plant pest risk. These sequences do not code for a protein and are not implicated in disease pathogenesis.

B. MS3 CORN HAS NO SIGNIFICANT POTENTIAL TO BECOME 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 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. Domesticated corn has also lost its perennial nature and its ability 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.

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 MS3 Corn in laboratory, greenhouse, and field tests support the conclusion that MS3 Corn has little potential to become a weed pest. With the exception of the tolerance to glufosinate, MS3 Corn has agronomic traits similar to those of traditionally bred male-sterile corn and does not exhibit traits that cause concern that they might become weed pests.

C. MS3 CORN WILL NOT INCREASE THE WEEDINESS POTENTIAL OF ANY OTHER PLANT WITH WHICH IT CAN INTERBREED.

APHIS considered whether the movement of the male sterility gene and the other genes from MS3 Corn to other cultivated corn or wild relatives might result in offspring that would present problems as weeds. First, the MS3 Corn is male sterile, thus significantly

reducing the likelihood of outcrossing. Second, 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 trait will be inadvertently incorporated into the germplasm of cultivated corn lines (Doebley, 1984).

D. MS3 CORN WILL NOT HARM ORGANISMS BENEFICIAL TO AGRICULTURE OR ORGANISMS THAT ARE DESIGNATED AS THREATENED OR ENDANGERED.

APHIS evaluated the potential for MS3 Corn 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 MS3 Corn or their progeny will exert any deleterious effects on organisms recognized as beneficial to agriculture. Likewise, cultivation of MS3 Corn will not harm any species designated as threatened or endangered. MS3 Corn produces a two enzymes, barnase and phosphinothricin acetyltransferase, that is not produced in nontransgenic corn. There is no indication that these enzymes are toxic to beneficial organisms or result in the production of toxic constituents. In addition, APHIS can envision no plausible mechanism whereby MS3 Corn would be injurious or pathogenic to beneficial organisms such as bees and earthworms.

The definition of MS3 Corn encompasses not only the corn lines that have already been field tested, but also new corn lines produced through conventional breeding using MS3 Corn as one or both parents. APHIS believes that the analysis applied to the MS3 Corn plants already field tested will apply equally well to these new corn lines, and that the data provided by Plant Genetic Systems USA Company justify the conclusion that such new lines derived from MS3 Corn will not present a plant pest risk. The variation in agronomic characteristics among the MS3 Corn 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 MS3 Corn will possess plant pest properties.

E. MS3 CORN SHOULD NOT CAUSE DAMAGE TO PROCESSED AGRICULTURAL COMMODITIES.

The characteristics of MS3 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 MS3 Corn exhibited the typical agronomic characteristics of the parent corn. In APHIS' opinion, the components and processing characteristics of MS3 Corn reveal no differences in any component that could have an indirect plant pest effect on any processed plant commodity.

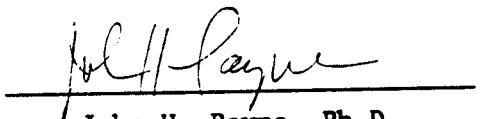
IV. CONCLUSION

APHIS has determined that MS3 Corn that have previously been field tested under permit will no longer be considered regulated articles under APHIS regulations at 7 CFR Part 340. Permits 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 MS3 Corn and vegetative plant material or seeds capable of propagation are still subject to the restrictions 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 MS3 Corn:

(1) exhibits no plant pathogenic properties; (2) is no more likely to become a weed than corn lines developed by traditional breeding techniques; (3) is unlikely to increase the weediness potential of any other cultivated plant or native wild species with which MS3 Corn can interbreed; (4) will not harm threatened or endangered species or other organisms, such as bees, which are beneficial to agriculture; and (5) will not cause damage to processed agricultural commodities.

APHIS has also concluded that there is a reasonable certainty that new progeny of MS3 Corn or varieties bred from these lines will not exhibit new plant pest properties, i.e., properties substantially different from any observed for MS3 Corn plants already field tested, or those observed for corn in traditional breeding programs.



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