

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. 95-023-2]

Availability of Determination of Nonregulated Status for Genetically Engineered Cotton

AGENCY: Animal and Plant Health Inspection Service, USDA.

ACTION: Notice.

SUMMARY: We are advising the public of our determination that the Monsanto Company's cotton lines designated as 1445 and 1698 that have been genetically engineered for tolerance to the herbicide glyphosate are no longer considered regulated articles under our regulations governing the introduction of certain genetically engineered organisms. Our determination is based on our evaluation of data submitted by the Monsanto 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 Monsanto 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: July 11, 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. Sivramiah Shantharam, 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 February 14, 1995, the Animal and Plant Health Inspection Service (APHIS) received a petition (APHIS Petition No. 95-045-01p) from the Monsanto Company (Monsanto) of St. Louis, MO, seeking a determination that cotton lines designated as 1445 and 1698 that have been genetically engineered for tolerance to the herbicide glyphosate do not present a plant pest risk and, therefore, are not regulated articles under APHIS' regulations in 7 CFR part 340.

On March 30, 1995, APHIS published a notice in the *Federal Register* (60 FR 16428-16430, Docket No. 95-023-1) announcing that the Monsanto 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 cotton lines and food products derived from them. In the notice, APHIS solicited written comments from the public as to whether the subject cotton lines posed a plant pest risk. The comments were to have been received by APHIS on or before May 30, 1995.

APHIS received a total of 10 comments on the Monsanto petition, from universities, cooperative extension service offices, agricultural experiment stations, a council representing cotton interests, and a State department of agriculture. All the commenters supported the Monsanto petition for nonregulated status for the subject cotton lines.

Analysis

Cotton lines 1445 and 1698 contain the gene for CP4 EPSPS (5-enolpyruvylshikimate-3-phosphate synthase) isolated from *Agrobacterium* sp. strain CP4; which encodes an enzyme conferring tolerance to glyphosate, the active ingredient in Roundup® herbicide. The subject cotton lines also contain the *nptII* gene, which

encodes the selectable marker neomycin phosphotransferase II. Cotton lines 1445 and 1698 were produced through the use of *Agrobacterium tumefaciens* transformation.

The subject cotton lines were considered regulated articles because they contain certain gene sequences (vectors, vector agents, promoters, and terminators) derived from plant pathogens. However, evaluation of field data reports from field tests of the subject cotton lines 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 cotton plants' release into the environment.

Determination

Based on its analysis of the data submitted by Monsanto and a review of other scientific data, comments received from the public, and field tests of the subject cotton lines, APHIS has determined that cotton lines 1445 and 1698: (1) Exhibit no plant pathogenic properties; (2) are no more likely to become weeds than cotton 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 cotton varieties derived from cotton lines 1445 and 1698 will exhibit new plant pest properties, i.e., properties substantially different from any observed for the cotton lines 1445 and 1698 already field tested, or those observed for cotton in traditional breeding programs.

The effect of this determination is that cotton lines designated as 1445 and 1698 are no longer considered regulated articles 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 cotton lines 1445 and 1698 or their progeny. However, the importation of the subject cotton lines 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 cotton lines 1445 and 1698 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 14th day of July 1995.

Lonnie J. King,

*Administrator, Animal and Plant Health
Inspection Service.*

[FR Doc. 95-18071 Filed 7-21-95; 8:45 am]

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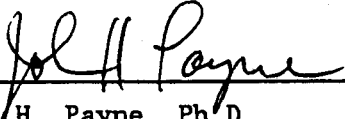


Monsanto Petition 95-045-01p to USDA/APHIS for Determination of
Nonregulated Status of Glyphosate Tolerant Cotton (Roundup Ready™)
Lines 1445 and 1698

Environmental Assessment and
Finding of No Significant Impact

July 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 cotton known as glyphosate tolerant cotton (Roundup Ready™) lines 1445 and 1698. APHIS received a petition from Monsanto Company regarding the status of cotton lines 1445 and 1698 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 on the analysis documented in this environmental assessment, APHIS has reached a finding of no significant impact on the environment from its determination that glyphosate tolerant cotton (Roundup Ready™) lines 1445 and 1698 shall no longer be regulated articles.



John H. Payne, Ph.D.
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Biotechnology, Biologics, and Environmental Protection
Animal and Plant Health Inspection Service
U.S. Department of Agriculture
Date: JUL 11 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) to decide whether or not the glyphosate tolerant (Roundup Ready™) cotton lines 1445 and 1698 (hereafter referred to as lines 1445 and 1698) should continue to be a regulated article under 7 CFR 340. Monsanto Company (hereafter referred to as Monsanto) petitioned APHIS requesting a determination on 1445 and 1698, and any progeny derived from them, as non-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 claim that lines 1445 and 1698 do not present a plant pest risk and therefore, should no longer be regulated articles under the APHIS regulations. Lines 1445 and 1698 have been considered regulated articles because they were engineered with DNA sequences derived from the plant pathogenic sources. As regulated articles, APHIS permits and notifications have been required for introductions (importation, interstate movements, and field tests) of 1445 and 1698.

Lines 1445 and 1698 were developed by using recombinant DNA techniques to introduce a gene for EPSPS (5-enolpyruvylshikimate-3-phosphate synthase), isolated from *Agrobacterium* sp. strain CP4 that encodes an enzyme which is naturally tolerant to glyphosate, the active ingredient of Roundup® herbicide. In addition, the *nptII* (neomycin phosphotransferase II) gene isolated from the bacterial transposon Tn5 that codes for resistance to the antibiotic kanamycin to function as a selectable marker in plants has been introduced into lines 1445 and 1698. The transgenic cotton lines that are subject of the petition were developed by a widely used technique called Agro-infection which essentially involves using a plant pathogenic strain of *Agrobacterium tumefaciens* and its disarmed plasmid vector.

Roundup® herbicide contains the active ingredient glyphosate which is a non-selective, post-emergent weed control agent. The target site of action of glyphosate is EPSPS that is present in all plants, bacteria, and fungi as a component of the Shikimate pathway of aromatic amino acid biosynthesis (Levin and Sprinson, 1994). The CP4 EPSPS which is naturally resistant to the inhibition by glyphosate (Padgett et al. 1993) has been introduced into 1445 and 1698 to confer tolerance to the foliar application of glyphosate.

Separate EAs were prepared by APHIS before granting the permits for every field trial with 1445 and 1698. Previous EAs (91-347-01, and 93-012-02, and 93-012-03 have addressed questions pertinent to plant pest risk issues relevant to the conduct of field trials under physical and reproductive confinement, but they do not address several issues that are relevant to the unconfined cultivation, and notifications 93-223-02n, 93-210-02n, 94-027-02n, and 94-273-03n were approved based on those EAs. With respect to these new issues, APHIS concludes the following:

1. Lines 1445 and 1698 exhibit no plant pathogenic properties. Although DNA sequences from a plant pathogen were used in their development, these cotton plants are not infected nor can these plants incite disease in other plants.
2. Lines 1445 and 1698 are no more likely to become weeds than cotton developed by traditional breeding techniques. Cotton is not considered to be a serious, principal or common weed pest in the U.S.
3. Lines 1445 and 1698 are unlikely to increase the weediness potential for any other cultivated or wild species with which they can interbreed. The introgression of the *EPSPS* gene from 1445 and 1698 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. Lines 1445 and 1698 will not harm other organisms, including agriculturally beneficial organisms and threatened and endangered species.
5. Lines 1445 and 1698 should not cause damage to processed agricultural commodities. Seeds of 1445 and 1698 are substantially equivalent in composition, quality, and other characteristics to nontransgenic cotton plants and should have no adverse impacts on raw or processed agricultural commodities.

Therefore, after a review of the available evidence, APHIS believes that 1445 and 1698 will be just as safe to grow as traditionally bred cotton 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 1445 and 1698 or their progeny were no longer considered regulated articles under the regulations.

II. BACKGROUND

Development of 1445 and 1698. In a petition dated February 9, 1995 Monsanto Company requested a determination from APHIS that 1445 and 1698, and any progeny derived from them, should no longer be considered regulated articles under APHIS regulations found at 7 CFR Part 340. 1445 and 1698 have been considered regulated articles because they were engineered with DNA sequences derived from the plant pathogenic sources, and the transformation employed *Agrobacterium tumefaciens*.

Transgenic cotton lines 1445 and 1698 were developed by using recombinant DNA techniques to introduce a gene for EPSPS (5-enolpyruvylshikimate-3-phosphate synthase), isolated from *Agrobacterium* sp. strain CP4 that encodes an enzyme which is naturally tolerant to glyphosate, the active ingredient of Roundup® herbicide. In addition, *nptII* (neomycin phosphotransferase II) gene isolated from bacterial transposon Tn5 that codes for resistance to the antibiotic kanamycin to function as a selectable marker in plants, and another selectable marker gene *aad* (3"(9)-O-aminoglycoside adenyltransferase (AAD) to function in bacterial cells to confer resistance to streptomycin and spectinomycin antibiotics have also been introduced into lines 1445 and 1698, but not expressed. The transgenic cotton lines that are the subject of the petition were developed by the use of a plant pathogenic strain of *Agrobacterium tumefaciens* and its disarmed plasmid vector.

Roundup herbicide contains the active ingredient glyphosate which is a non-selective, post-emergent weed control agent. The target site of action of glyphosate is EPSPS that is present in all plants, bacteria, and fungi as a component of the Shikimate pathway of aromatic amino acid biosynthesis (Levin and Sprinson, 1994). The CP4 EPSPS which is naturally resistant to the inhibition by glyphosate (Padgett et al. 1993) has been introduced into 1445 and 1698 to confer tolerance to the foliar application of glyphosate.

1445 and 1698 have been field tested since 1991 in the major cotton growing regions of the United States under permits and acknowledgements of notifications by APHIS (USDA Permit Numbers 91-347-01 in Alabama, and 93-012-02 in Alabama, Arkansas, Arizona, California, Georgia, Louisiana, Mississippi, and Texas, 93-012-02 in North Carolina, and notifications 93-223-02n and 93-210-02n in Puerto Rico, 94-027-01n in Alabama, Arkansas, Arizona, California, Georgia, Louisiana, Mississippi, Tennessee, and Texas, 94-027-02n in North Carolina, and 94-273-03n in Alabama, Louisiana, and Texas). Lines 1445 and 1698 have been evaluated extensively in laboratory, greenhouse, and field experiments to confirm that they exhibit the desired agronomic characteristics and do not present a plant pest risk. Although the field tests of 1445 and 1698 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. 1445 and 1698 are also subject to regulation by other agencies. APHIS' decision on the regulatory status of 1445 and 1698 under APHIS' regulations at 7 CFR 340, does not release this cotton and its progeny from EPA and 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 1445 and 1698 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 determining the status of 1445 and 1698 as regulated articles under APHIS regulations. The developer of 1445 and 1698, Monsanto Company, submitted a petition to APHIS requesting that APHIS make a determination that 1445 and 1698 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 1445 and 1698 are no longer regulated articles under the regulations at 7 CFR Part 340. Permits from APHIS would still be required for introductions of 1445 and 1698. APHIS might choose this alternative if there were insufficient evidence to demonstrate the lack of plant pest risk from uncontained cultivation of 1445 and 1698.

B. Determination that 1445 and 1698 is no longer a regulated article.

Under this alternative, 1445 and 1698 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 1445 and 1698. A basis for this determination would include a "Finding of No Significant Impact" under the NEPA.

V. AFFECTED ENVIRONMENT AND POTENTIAL ENVIRONMENTAL IMPACTS

This EA addresses potential environmental impacts from an APHIS determination that 1445 and 1698 should no longer be considered regulated articles. Previous EAs (91-347-01, and 93-012-02, and 93-012-03 have addressed questions pertinent to plant pest risk issues relevant to the conduct of field trials under physical and reproductive confinement, but they do not address several issues that are relevant to the unconfined cultivation, and notifications 93-223-02n, 93-210-02n, 94-027-02n, and 94-273-03n were approved based on those EAs. This EA discusses the genetic modifications and the potential environmental impacts that might be associated with the unconfined cultivation of 1445 and 1698.

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 cotton, the genetic components used in the construction of 1445 and 1698, and the analyses that lead APHIS to conclude that 1445 and 1698 have no potential to present 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 transformed lines 1445 and 1698

Transgenic cotton lines 1445 and 1698 were developed by using recombinant DNA techniques to introduce a gene for EPSPS (5-enolpyruvylshikimate-3-phosphate synthase), isolated from *Agrobacterium* sp. strain CP4 that encodes an enzyme which is naturally tolerant to glyphosate, the active ingredient of Roundup® herbicide. In addition, *nptII* (neomycin phosphotransferase II) gene isolated from bacterial transposon Tn5 that codes for resistance to the antibiotic kanamycin to function as a selectable marker in plants, and another selectable marker gene *aad* (3"(9)-O-aminoglycoside adenyltransferase (AAD) to function in bacterial cells to confer resistance to streptomycin and spectinomycin antibiotics but does not express have also been introduced into lines 1445 and 1698. The transgenic cotton lines that are the subject of the petition were developed by a widely used technique called Agro-infection which essentially involves using a plant pathogenic strain of *Agrobacterium tumefaciens* and its disarmed plasmid vector.

Roundup® herbicide contains the active ingredient glyphosate which is a non-selective, post-emergent weed control agent. The target site of action of glyphosate is EPSPS that is present in all plants, bacteria, and fungi as a component of the Shikimate pathway of aromatic amino acid biosynthesis (Levin and Sprinson, 1994). The CP4 EPSPS which is naturally resistant to the inhibition by glyphosate (Padgett *et al.* 1993) has been introduced into 1445 and 1698 to confer tolerance to the foliar application of glyphosate.

It is amply clear from our previous EAs and also from the scientific evidence in the published literature that even though several DNA components and live bacteria were used to develop these transgenic cotton lines, none of these components from plant pathogenic organisms have any inherent ability to incite disease in the transformed plants as they have either been disarmed or the bacteria have been killed with a potent antibiotic. As such they do not present a risk of plant pests being introduced into the environment by way of uncontained cultivation of these transgenic cotton lines 1445 and 1698.

B. Potential for 1445 and 1698 to become successful weeds

Cotton 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, cotton 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 1445 and 1698 is a line of cotton (*Gossypium hirsutum* L.) known as Coker 312 that exhibits no appreciable weedy characteristics. The EPSPS gene is unlikely to increase weediness of 1445 and 1698. The glyphosate resistance of these plants will confer a selective advantage only when glyphosate is applied to the plants. No other attributes of 1445 and 1698 suggest that it be any more "weedy" than traditionally-bred cotton cultivars. Other than the resistance to the herbicide glyphosate, 1445 and 1698 have retained the agronomic characteristics of the parental cotton, including the sensitivity to other herbicides.

Monsanto 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 1445 and 1698 are no more likely to become weeds than cotton developed by traditional breeding techniques.

C. Potential for 1445 and 1698 to increase the weediness potential of any other plant with which it can interbreed.

Cotton belongs to the genus *Gossypium* of the tribe *Gossypeae* of the family Malvaceae (Fryxell, 1979; Munro, 1987). Only four species of cotton are of any agronomic importance in the world; two diploid old world cotton or Asiatic cotton and two allotetraploid New World species. The old world cotton is restricted to India, Africa and Asia (Munro, 1987). But, the new world cotton comprises of 98% of cotton cultivated for fibre production. Wild species of cotton occur in arid parts of the tropics and subtropics. Fryxell (1984) has divided the wild diploid species into three geographical groups: the Australian group (11 species), the Afro-Arabian group (8 species), and the American group (12 species). Two species of the American group (wild tetraploid) occur in Peru and in the Galapagos, and the remaining 10 occur in Western Mexico with one (*G. thurberi* Todaro) extending up to Arizona. *G. tomentosum* and *G. hirsutum* are two of the new world cottons that occur in Hawaii and middle America and drier areas of southern tip of Florida (Fryxell, 1984; Lee, 194). Wild populations of *G. hirsutum* are relatively rare and tend to be widely dispersed as beach strands or on small islands. There are examples of escaped cotton belonging to *G. hirsutum* and *G. barbedense* growing in the wild in Southern Florida and Hawaii. These escaped plants appear opportunistic toward disturbed land and appear not be effective in inhabiting managed ecosystems.

Although natural outcrossing can occur in cotton, it is normally self-pollinating (Niles and Feaster, 1984). The pollen is heavy and sticky, and is heavily pollinated via bumble bees and honey bees. The range of natural crossing is very limited (100-200 feet) (McGregor, 1976).

APHIS considered whether the movement of the *EPSPS* gene from 1445 and 1698 to other cultivated cotton or wild relatives might result in offspring that would present problems as weeds. The genetic integrity of commercial cultivated cotton lines and varieties is strictly controlled through established plant breeding practices. These standard practices make it unlikely that this glyphosate tolerance trait will be inadvertently incorporated into the germplasm of cultivated cotton lines.

D. Potential for 1445 and 1698 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 1445 and 1698 to directly or indirectly harm other organisms, including those that are recognized as beneficial to agriculture and to those that are recognized as threatened or endangered in the United States.

APHIS concluded that the available evidence suggests that 1445 and 1698 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 glyphosate herbicides in the cultivation of 1445 and 1698, 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 1445 and 1698 to damage agricultural commodities.

APHIS can envision no way in which 1445 and 1698 would damage agricultural commodities. With the exception of two enzymes, *EPSPS* and *NPTII*, the composition and attributes of 1445 and 1698 are indistinguishable from the parental line of cotton used to develop 1445 and 1698. There is no indication that the *EPSPS* enzyme itself will affect the qualities of commodities derived from 1445 and 1698.

VI. CONCLUSION

APHIS has evaluated information from the scientific literature as well as information submitted by Monsanto Company that characterized 1445 and 1698. After careful analysis, APHIS has identified no significant impact to the environment from issuance of a determination that 1445 and 1698 should no longer be regulated articles under APHIS regulations at 7 CFR Part 340. This finding is supported by the following conclusions:

1. Lines 1445 and 1698 exhibit no plant pathogenic properties. Although DNA sequences from a plant pathogen were used in their development, these cotton plants are not infected nor can these plants incite disease in other plants.
2. Lines 1445 and 1698 are no more likely to become weeds than cotton developed by traditional breeding techniques. Cotton is not considered to be a serious, principal or common weed pest in the U.S.
3. Lines 1445 and 1698 are unlikely to increase the weediness potential for any other cultivated or wild species with which they can interbreed. The introgression of the *EPSPS* gene from 1445 and 1698 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. Lines 1445 and 1698 will not harm other organisms, including agriculturally beneficial organisms and threatened and endangered species.
5. Lines 1445 and 1698 should not cause damage to processed agricultural commodities. Seeds of 1445 and 1698 are substantially equivalent in composition, quality, and other characteristics to nontransgenic yellow dent cotton and should have no adverse impacts on raw or processed agricultural commodities.

Therefore, after review of the available evidence, APHIS concludes that 1445 and 1698 will be just as safe to grow as traditionally-bred cotton 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 1445 and 1698 were no longer considered regulated articles under its regulations at 7 CFR Part 340.

VII. LITERATURE CITED

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VIII. PREPARERS AND REVIEWERS

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Response to Monsanto Company Petition 95-045-01p For a Determination
of Nonregulated Status for Glyphosate Tolerant (Roundup Ready™)
Cotton Lines 1445 and 1698

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

I. SUMMARY

In a petition dated February 9, 1995, Monsanto Company requested a determination from the Animal and Plant Health Inspection Service (APHIS) that glyphosate tolerant cotton lines 1445 and 1698, and any progeny derived from them, should no longer be considered regulated articles under APHIS regulations 7 CFR Part 340. The glyphosate tolerant cotton lines 1445 and 1698 (hereafter called 1445 and 1698) have been considered regulated articles because they were engineered with DNA sequences derived from the plant pathogens, caulimoviruses and *Agrobacterium* sp. strain CP4.

Lines 1445 and 1698 were developed by using recombinant DNA techniques to introduce a modified version of a *EPSPS* gene, which encodes the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (*EPSPS*). The *EPSPS* introduced into these transgenic cotton lines is a resistant form of the similar enzyme present in all plants, bacteria and fungi, and thereby confer resistance or tolerance to the herbicide glyphosate. The *EPSPS* gene was originally isolated from the common soil borne plant pathogenic microorganism, *Agrobacterium* sp. strain CP4. After isolation, the *EPSPS* gene was modified by (1) attaching noncoding DNA regulatory sequences from a caulimovirus and (2) altering codon usage of the *EPSPS* coding region to enhance expression of the *EPSPS* gene in plants. In addition, the *nptII* (neomycin phosphotransferase II) gene isolated from bacterial transposon Tn5 that codes for resistance to the antibiotic kanamycin to function as a selectable marker in plants, and another selectable marker gene *aad* (3"(9)-O-aminoglycoside adenylyltransferase (AAD) to function in bacterial cells to confer resistance to streptomycin and spectinomycin antibiotics have also been introduced into pedigreed cotton Coker 312 lines 1445 and 1698, but not expressed in them. The transgenic cotton lines that are subject of the petition were developed by a widely used technique called Agro-infection which essentially involves using a plant pathogenic strain of *Agrobacterium tumefaciens* and its disarmed plasmid vector.

Roundup® herbicide contains the active ingredient glyphosate which is a non-selective, post-emergent weed control agent. The target site of action of glyphosate is *EPSPS* that is present in all plants, bacteria, and fungi as a component of the Shikimate pathway of aromatic amino acid biosynthesis (Levin and Sprinson, 1964). The CP4 *EPSPS* which is naturally resistant to the inhibition by glyphosate (Padgett *et.al.* 1993) has been introduced into 1445 and 1698 to confer tolerance to the foliar application of glyphosate.

Based on a review of available scientific information, APHIS has determined that 1445 and 1698 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, regulatory oversight under these regulations will no longer be required from APHIS for field testing, importation, or interstate movement of 1445 and 1698 or their progeny.

This determination has been made based on an analysis that revealed that 1445 and 1698: (1) exhibit no plant pathogenic properties; (2) are no more likely to become weeds than cotton 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, threatened and endangered organisms, or organisms such as bees, which are beneficial to cotton cultivation and agriculture in general; and 5) do not cause damage to processed agricultural commodities. APHIS has also concluded that there is no reason to believe that new progeny cotton varieties derived from 1445 and 1698 will exhibit new plant pest properties, i.e., properties substantially different from any observed for the 1445 and 1698 cotton lines already field tested, or those observed for cotton 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. Lines 1445 and 1698 have been considered "regulated articles" under Part 340 of the regulations because they have been engineered with certain noncoding regulatory and vector DNA sequences derived from the plant pathogenic organisms like the caulimoviruses, and an *Agrobacterium* species.

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 1445 and 1698 under regulations 7 CFR 340, does not release these cotton plants and their progeny from EPA and FDA regulatory oversight. The regulation of herbicide use on these cotton lines is under the jurisdiction of the EPA.

III. PUBLIC COMMENTS

APHIS received a total of ten comments on the petition from the following: a state department of agriculture (1); commodity group (1) and universities (8). All comments were strongly supportive of the petition. There was not a single negative comment received on the petition.

IV. ANALYSIS OF 1445 and 1698

Biology of Cotton: Cotton belongs to the genus *Gossypium* of the tribe *Gossypieae* of the family Malvaceae (Fryxell, 1979; Munro, 1987). Only four species of cotton are of any agronomic importance in the world; two diploid old world cotton or Asiatic cotton and two allotetraploid New World species. The old world cotton is restricted to India, Africa and Asia (Munro, 1987). The new World cotton comprises of 98% of cotton cultivated for fibre production. Wild species of cotton occur in arid parts of the tropics and subtropics. Fryxell (1984) has divided the wild diploid species into three geographical groups: the Australian group (11 species), the Afro-Arabian group (8 species), and the American group (12 species). Two species of the American group (tetraploid cotton) occur in Peru and in the Galapagos, and the remaining 10 occur in Western Mexico with one (*G. thurberi* Todaro) extending up to Arizona. *G. tomentosum* and *G. hirsutum* are two of the New World cottons that occur in Hawaii and middle America and drier areas of southern tip of Florida (Fryxell, 1984; Lee, 194). Wild populations of *G. hirsutum* are relatively rare and tend to be widely dispersed as beach strands or on small islands. There are examples of escaped cotton belonging to *G. hirsutum* and *G. barbedense* growing in the wild in Southern Florida and Hawaii. These escaped plants appear opportunistic toward disturbed land and appear not to be effective in inhabiting managed ecosystems.

Although natural outcrossing can occur in cotton, they are normally self-pollinating (Niles and Feaster, 1984). The pollen is heavy and sticky, and is heavily pollinated via bumble bees and honey bees. The range of natural crossing is very limited (100-200 feet) (McGregor, 1976).

APHIS considered whether the movement of the *EPSPS* gene from 1445 and 1698 to other cultivated cotton or wild relatives might result in offspring that would present problems as weeds. The genetic integrity of commercial cultivated cotton lines and varieties is strictly controlled through established plant breeding practices. These standard practices make it unlikely that this glyphosate tolerance

trait will be inadvertently incorporated into the germplasm of cultivated cotton lines.

Rationale for Development of Glyphosate tolerant Cotton: Weeds are a severe constraint for cotton production specially when young cotton seedlings cannot compete with aggressive weeds in the early stages of seedling establishment. Current weed removal practices are inadequate at best to provide a weed free environment. In the United States alone, the cotton crop loss due to weeds is estimated to be \$400 million annually. Weed management is a critical factor for cotton 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. For years, pre-emergence herbicides have been the major tool used for weed control in conventional cotton 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 cotton, 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 Roundup® as an effective post emergence herbicide with glyphosate tolerant cotton may make it possible to reduce the use of pre-emergence herbicides in cotton production.

Roundup® herbicide contains the active ingredient glyphosate which is a non-selective, post-emergent weed control agent. The target site of action of glyphosate is EPSPS that is present in all plants, bacteria, and fungi as a component of the Shikimate pathway of aromatic amino acid biosynthesis (Levin and Sprinson, 1964). The CP4 EPSPS which is naturally resistant to the inhibition by glyphosate (Padgett *et al.* 1993) has been introduced into 1445 and 1698 to confer tolerance to the foliar application of glyphosate. Roundup has the advantage of low residual activity, low soil leaching, and low toxicity to nontarget organisms or development of resistant weeds (Atkinson, 1985). Roundup toxicity recorded on certain fishes and invertebrates is due to the surfactant present in commercial formulations (Atkinson, 1985). Certain soil invertebrates show minor toxicity to glyphosate (Eijsackers, 1985). Although there are reported instances of certain cellular activities of a select group of microbes, they are of no undue concern as the commercial applications of glyphosate rarely exceed concentrations that cause the said effects (Carlisle and Trevors, 1988). EPA has classified glyphosate under Category E (evidence of non-carcinogenicity from humans) 57 FR 8739. Roundup is readily degraded by microorganisms in the soil.

Development of 1445 and 1698:

Lines 1445 and 1698 were developed by using recombinant DNA techniques to introduce a modified version of a *EPSPS* gene, which encodes the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS). The EPSPS introduced into these transgenic cotton lines is a resistant form of the similar enzyme present in all plants, bacteria and fungi, and thereby confer resistance or tolerance in the transgenic plants. The *EPSPS* gene was originally isolated from the common soil borne plant pathogenic microorganism, *Agrobacterium* sp. strain CP4. After isolation, the *EPSPS* gene was modified by (1) attaching noncoding DNA regulatory sequences from a Caulimovirus and (2) altering codon usage of the *EPSPS* coding region to enhance expression of the *EPSPS* gene in plants. In addition, *nptII* (neomycin phosphotransferase II) gene isolated from bacterial transposon Tn5 that codes for resistance to the antibiotic kanamycin to function as a selectable marker in plants, and another selectable marker gene *aad* (3''(9)-O-aminoglycoside adenyltransferase (AAD) to function in bacterial cells to confer resistance to streptomycin and spectinomycin antibiotics have also been introduced into pedigreed cotton Coker 312 lines 1445 and 1698, but not expressed in them. The transgenic cotton lines that are subject of the petition were developed by a widely used technique called Agro-infection which essentially involves using a plant pathogenic strain of *Agrobacterium tumefaciens* and its disarmed plasmid vector (Umbeck et al. 1987; Trolinder and Goodin, 1987).

EPSPS is commonly found in plants, bacteria and fungi, and there has been no recorded instance of this enzyme causing toxicity to any living species of organism. The enzyme degrades rapidly in the environment upon natural decay of the vegetative parts of the plants.

Construction of the Binary Vectors Used in Transformation. The plasmids PV-GHGT06 and PV-GHGT07 used to transform the Coker 312 variety of cotton in tissue culture have Ti-plasmid backbone derived from *A. tumefaciens*. These plasmids are based on the incompatibility group P (IncP) plasmids referred to as RK2 (Stalker et al. 1981), and contain the synthetic *EPSPS* gene regulated by a caulimovirus promoter and terminator sequences derived from *A. tumefaciens*. In addition, the plasmid contains an streptomycin/spectinomycin resistance (*aad* 3'') gene and a bacterial origin of replication. The *aad*3'' gene has regulatory sequences recognized only in bacteria but not functional in the transgenic cotton cells. Therefore, the *EPSPS* and *nptII* are the only introduced genes which can be expressed in the plant cells. The vectors have only one of the border sequences of the Ti-Plasmid from integration into the plant DNA.

Protoplast Transformation System: The transgenic cotton lines that are subject of the petition were developed by a widely used technique called Agro-infection which essentially involves using a plant pathogenic strain of *Agrobacterium tumefaciens* and its disarmed plasmid vector (Umbeck et al. 1987; Trolinder and Goodin, 1987).

A. THE INTRODUCED GENES, THEIR PRODUCTS, AND THE ADDED REGULATORY SEQUENCES CONTROLLING THEIR EXPRESSION DO NOT PRESENT A PLANT PEST RISK IN 1445 and 1698.

Southern blot analyses indicate that 1445 and 1698 contain 1 and 2 copies of the *EPSPS* gene, respectively. Once inserted into the chromosome of the cotton plant, the introduced *EPSPS* gene is maintained and transmitted in the same manner as any other genes following the Mendelian laws of inheritance.

Expression of the *EPSPS* gene in 1445 and 1698 is modulated by noncoding DNA regulatory sequences derived from the plant pathogens. Specifically, these regulatory sequences are the promoter and 3'-nontranslated regions of the caulimoviruses (Gowda et al. 1989). These regulatory sequences are utilized widely in the expression of genes engineered into plants. Although these regulatory sequences are derived from plant pathogens, there is no evidence to suggest that they pose a plant pest risk.

B. 1445 and 1698 HAVE NO SIGNIFICANT POTENTIAL TO BECOME SUCCESSFUL WEEDS.

Cotton 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, cotton 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 1445 and 1698 is a line of cotton (*Gossypium hirsutum* L.) known as Coker 312 that exhibits no appreciable weedy characteristics. The *EPSPS* gene is unlikely to increase weediness of 1445 and 1698. The glyphosate resistance of these plants will confer a selective advantage only when glyphosate is applied to the plants. No other attributes of 1445 and 1698 suggest that it be any more "weedy" than traditionally-bred cotton cultivars. Other than the resistance to the herbicide glyphosate, 1445 and 1698 have retained the agronomic characteristics of the parental cotton, including the sensitivity to other herbicides.

Monsanto 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 1445 and 1698 are no more likely to become weeds than

cotton developed by traditional breeding techniques. Cotton is not considered a weed.

A weed pest is a plant that grows persistently in locations where it is unwanted. Cotton 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. Cotton is not classified as a serious, principal, or common weed pest (Holm et al., 1979). Cotton is considered a highly domesticated, well-characterized crop plant that is not persistent in undisturbed environments without human intervention.

Evaluations of 1445 and 1698 in laboratory, greenhouse, and field tests support the conclusion that 1445 and 1698 have little potential to become weed pests. With the exception of the resistance to glyphosate, 1445 and 1698 have agronomic traits similar to those of traditionally bred cotton and do not exhibit traits that cause concern that they might become weed pests.

C. 1445 and 1698 WILL NOT INCREASE THE WEEDINESS POTENTIAL OF ANY OTHER PLANT WITH WHICH IT CAN INTERBREED.

Cotton belongs to the genus *Gossypium* of the tribe *Gossypiae* of the family Malvaceae (Fryxell, 1979; Munro, 1987). Only four species of cotton are of any agronomic importance in the world; two diploid old world cotton or Asiatic cotton and two allotetraploid new world species. The old world cotton is restricted to India, Africa and Asia (Munro, 1987). But, the new World cotton comprises of 98% of cotton cultivated for fibre production. Wild species of cotton occur in arid parts of the tropics and subtropics. Fryxell (1984) has divided the wild diploid species into three geographical groups: the Australian group (11 species), the Afro-Arabian group (8 species), and the American group (12 species). Two species of the American group occur in Peru and in the Galapagos, and the remaining 10 occur in Western Mexico with one (*G. thurberi* Todaro) extending up to Arizona. *G. tomentosum* and *G. hirsutum* are two of the New World cottons that occur in Hawaii and middle America and drier areas of southern tip of Florida (Fryxell, 1984; Lee, 194). Wild populations of *G. hirsutum* are relatively rare and tend to be widely dispersed as beach strands or on small islands. There are examples of escaped cotton belonging to *G. hirsutum* *G. barbedense* growing in the wild in Southern Florida and Hawaii. These escaped plants appear opportunistic toward disturbed land and appears not be effective in inhabiting managed ecosystems.

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APHIS considered whether the movement of the *EPSPS* gene from 1445 and 1698 to other cultivated cotton or wild relatives might result in offspring that would present problems as weeds. The genetic integrity of commercial cultivated cotton lines and varieties is strictly controlled through established plant breeding practices. These standard practices make it unlikely that this glyphosate tolerance trait will be inadvertently incorporated into the germplasm of cultivated cotton lines.

D. Lines 1445 and 1698 WILL NOT HARM ORGANISMS BENEFICIAL TO AGRICULTURE OR ORGANISMS THAT ARE DESIGNATED AS THREATENED OR ENDANGERED.

APHIS evaluated the potential for 1445 and 1698 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 1445 and 1698 cotton or their progeny will exert any deleterious effects on organisms recognized as beneficial to agriculture. Likewise, cultivation of 1445 and 1698 will not harm any species designated as threatened or endangered. Lines 1445 and 1698 produce two enzymes, *EPSPS* and *NPTII*, that are not produced in nontransgenic cotton. 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 mechanism whereby 1445 and 1698 would be injurious or pathogenic to beneficial organisms such as bees and earthworms.

The definition of 1445 and 1698 encompasses not only the cotton lines that already have been field tested, but also new cotton lines produced through conventional breeding using 1445 and 1698 as one or both parents. APHIS believes that the analysis applied to the 1445 and 1698 plants already field tested will apply equally well to these new cotton lines, and that the data provided by Monsanto Company justify the conclusion that such new lines derived from 1445 and 1698 will not present a plant pest risk. The variation in agronomic characteristics among the 1445 and 1698 plants that have been field tested does not differ significantly from that seen in commercial cultivars of cotton that have never been considered regulated articles. Therefore, there is no reason to believe that any of the progeny of 1445 and 1698 will possess plant pest properties.

**E. 1445 and 1698 SHOULD NOT CAUSE DAMAGE TO PROCESSED AGRICULTURAL
COMMODITIES.**

The characteristics of 1445 and 1698 cotton 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 1445 and 1698 exhibited the typical agronomic characteristics of the parent cotton. In the opinion of APHIS, the components and processing characteristics of 1445 and 1698 reveal no differences in any component that could have an indirect plant pest effect on any processed plant commodity.

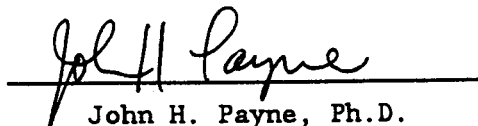
IV. CONCLUSIONS

APHIS has determined that 1445 and 1698 that previously have been field tested under permits and notification system will no longer be considered regulated articles under APHIS regulations found at 7 CFR Part 340. Notifications under those regulations will no longer be required from APHIS for field testing, importation, or interstate movement of these cottons or their progeny. However, the importation of 1445 and 1698 cotton 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) Lines 1445 and 1698 exhibit no plant pathogenic properties;
- 2) Lines 1445 and 1698 are no more likely to become weeds than cotton developed by traditional breeding techniques;
- 3) Lines 1445 and 1698 are unlikely to increase the weediness potential for any other cultivated or wild species with which they can interbreed;
- 4) Lines 1445 and 1698 will not harm other organisms, including agriculturally beneficial organisms and threatened and endangered species; and
- 5) Lines 1445 and 1698 should not cause damage to processed agricultural commodities.

APHIS has also concluded that there is a reasonable certainty that lines 1445 and 1698 or varieties bred from these lines will not exhibit new plant pest properties, i.e., properties substantially different from any observed for 1445 and 1698 plants already field tested, or those observed for cotton in traditional breeding programs.



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Date JUL 11 1995

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