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

DEPARTMENT OF AGRICULTURE

Animal and Plant Health Inspection Service

[Docket No. 95-075-2]

Dupont Agricultural Products; Availability of Determination of Nonregulated Status for Cotton Line Genetically Engineered for Tolerance to Sulfonylurea Herbicides

AGENCY: Animal and Plant Health Inspection Service, USDA.

ACTION: Notice.

SUMMARY: We are advising the public of our determination that a cotton line developed by Dupont Agricultural Products designated as 19-51a that has been genetically engineered for tolerance to sulfonylurea herbicides 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 Dupont Agricultural Products 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 Dupont Agricultural Products 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: January 25, 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. 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 September 13, 1995, the Animal and Plant Health Inspection Service (APHIS) received a petition (APHIS Petition No. 95-256-01p) from Dupont Agricultural Products (Dupont) of Wilmington, DE, seeking a determination that a cotton line designated as 19-51a that has been genetically engineered for tolerance to sulfonylurea herbicides does not present a plant pest risk and, therefore, is not a regulated article under APHIS' regulations in 7 CFR part 340.

On October 26, 1995, APHIS published a notice in the Federal Register (60 FR 54839-54840, Docket No. 95-075-1) announcing that the Dupont 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 line and food products derived from it. In the notice, APHIS solicited written comments from the public as to whether cotton line 19-51a posed a plant pest risk. The comments were to have been received by APHIS on or before December 26, 1995. APHIS received two comments on the subject petition during the designated 60-day comment period. Both comments were from State departments of agriculture and both were favorable to the petition.

Analysis

Cotton line 19-51a has been genetically engineered with a gene from tobacco which encodes an altered acetolactate synthase enzyme that enhances tolerance to sulfonylurea herbicides. The subject cotton line was developed through the use of the *Agrobacterium tumefaciens* transformation system.

Cotton line 19-51a has been considered a regulated article under APHIS' regulations in 7 CFR part 340 because it contains regulatory gene sequences derived from the plant pathogen *A. tumefaciens*. However, evaluation of field data reports from field tests of the subject cotton line conducted under APHIS permits or notifications since 1991 indicates that there were no deleterious effects on plants, nontarget organisms, or the environment as a result of the subject cotton plants' release into the environment.

Determination

Based on its analysis of the data submitted by Dupont and a review of other scientific data, comments received, and field tests of the subject cotton line, APHIS has determined that cotton line 19-51a: (1) Exhibits no plant pathogenic properties; (2) is no more likely to become a weed than cotton developed by traditional breeding techniques; (3) is unlikely to increase the weediness potential for any other cultivated or wild species with which it can interbreed; (4) will not harm other organisms, including agriculturally beneficial organisms and threatened and endangered species; and (5) should not cause damage to raw or processed agricultural commodities. Therefore, APHIS has concluded that cotton line 19-51a and any progeny derived from hybrid crosses with other nontransformed cotton varieties will be just as safe to grow as traditionally bred cotton lines that are not regulated under 7 CFR part 340.

The effect of this determination is that Dupont's cotton line designated as 19-51a 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 cotton line 19-51a or its progeny. However, the importation of the subject cotton 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 cotton line 19–51a 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 14th day of February 1996.

Terry L. Medley,

Administrator, Animal and Plant Health Inspection Service.

[FR Doc. 96–3824 Filed 2–20–96; 8:45 am]

BILLING CODE 3410–34–P

DuPont Petition 95-256-01p to USDA/APHIS for Determination of Nonregulated Status of Sulfonylurea Tolerant Cotton line 19-51a

Environmental Assessment and
Finding of No Significant Impact

January 1996

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 a genetically engineered cotton line designated as sulfonylurea tolerant cotton line 19-51a. APHIS received a petition from DuPont regarding the status of cotton line 19-51a as a regulated article under APHIS regulations at 7 CFR Part 340. APHIS has conducted a thorough review of the petition and its 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 sulfonylurea tolerant cotton line 19-51a shall no longer be a regulated article.

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Acting Director
Biotechnology, Biologics, and Environmental Protection
Animal and Plant Health Inspection Service
U.S. Department of Agriculture
Date: JAN 25 1996

I. SUMMARY

The Animal and Plant Health Inspection Service (APHIS) of the United States Department of Agriculture (USDA), has prepared an Environmental Assessment (EA) prior to making a determination on the regulatory status of a genetically engineered sulfonylurea tolerant cotton line designated as line 19-51a. Dupont Agricultural Products (Dupont) petitioned APHIS for a determination that line 19-51a does not present a plant pest risk and therefore, should no longer be a regulated article under the APHIS regulations at 7 CFR 340 (hereafter referred to as the regulations). As a regulated article under the regulations, APHIS permits and notifications have been required for introductions (importation, interstate movements, and environmental release) of line 19-51a.

Line 19-51a was developed by recombinant DNA techniques to introduce a chimeric tobacco gene that encodes an acetolactate synthase (ALS) enzyme which is tolerant to sulfonylurea. The transgenic cotton line that is the subject of the petition was developed by a widely used technique called Agro-infection which essentially involves using a plant pathogenic strain of *Agrobacterium tumefaciens* and its disarmed Ti-plasmid.

The sulfonylureas are a group of compounds which inhibit acetolactate synthase (ALS), the enzyme that catalyzes the first common step in the biosynthesis of the essential amino acids isoleucine, leucine, and valine (LaRossa and Schloss 1984, Falco 1989). These compounds inhibit plant growth by inactivating an enzyme in an essential amino acid biosynthetic pathway. Essential amino acids are not produced by mammals, and therefore they lack the target enzyme, contributing to the low mammalian toxicity of the sulfonylureas. The chimeric *S4-HrA* gene expresses an herbicide tolerant ALS (Chaleff and Mauvais 1984, Mazur *et al.* 1987) which allows the cotton plant to produce the essential amino acids in the presence of the sulfonylureas. The development of sulfonylurea tolerant plants has been documented previously (Mazur and Falco 1989, Haughn *et al.* 1988, Lee *et al.* 1988). ALS genes have been isolated from bacteria, fungi and plants (Friden *et al.* 1985, Falco *et al.* 1985, Mazur *et al.* 1987) and thus the enzyme is present in food derived from these sources. The deduced amino acid sequence from three ALS isozymes in *Escherichia coli* and *Salmonella typhimurium* has shown that these isozymes have three highly conserved regions between four non-conserved regions. Plant enzymes are also highly conserved within these domains but the highly conserved regions between plant enzymes extends through the entire length of the mature protein. Although the majority of the ALS enzymes found in nature are sensitive to inhibition by sulfonylureas there are examples of naturally tolerant ALS enzymes, such as the ALS isozyme I of *E. coli* (LaRossa and Smulski 1984).

Cotton line 19-51a was tested under APHIS field release permits at two sites in two states in 1991 (91-025-02), at six sites in three states in 1992 (91-358-01), at six sites in three locations in 1993 (93-053-01), under APHIS notification in the winter 1993/1994 in Puerto Rico (93-250-03N) and in 1994 under APHIS notification at approximately 19 locations in nine states (94-021-09N, 94-069-06N, 94-090-08N, 94-095-12N, 94-103-02N, 94-104-01N, 94-109-02N and 94-090-09N. In 1995, cotton line 19-51a has been or is currently being tested at approximately 50 sites in 14 states under APHIS notifications 95-026-01N, 95-066-07N, 95-066-08N, 95-088-01N, 95-060-04N, 95-066-09N and 95-066-10N. The data from these trials, results from laboratory experiments, and literature references demonstrate

that line 19-51a is not a plant pest, does not demonstrate any weediness potential greater than that seen in non-transgenic cotton, and does not have any selective advantage over non-transgenic cotton except in those instances where the cotton is treated with a herbicide which is active towards ALS. Composition analysis confirms that levels of important constituents of cotton seed from line 19-51a, including key antinutritional factors, are within the normal range for cotton. Cotton line 19-51a differs from other cotton varieties only in its resistance to sulfonylurea herbicides and Staple® herbicide.

Based on the submitted data, and published literature, APHIS concludes the following:

1. Line 19-51a exhibits no plant pathogenic properties. Although DNA sequences from a plant pathogen were used in their development, these cotton plants are neither infected nor can they cause disease in other plants.

2. Line 19-51a is no more likely to become a weed than cotton developed by traditional breeding techniques. Cotton is not a serious, principal or common weed pest in the United States of America.

3. Line 19-51a is unlikely to increase the weediness potential for any other cultivated or wild species with which it can interbreed. The introgression of the *ALS* gene from Line 19-51a into wild or cultivated sexually-compatible plants is extremely unlikely, and such rare events should not increase the weediness potential of any resulting progeny.

4. Line 19-51a will not harm other organisms, including agriculturally beneficial organisms and threatened and endangered species.

5. Line 19-51a should not cause damage to processed agricultural commodities. Seeds of line 19-51a 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 line 19-51a 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 line 19-51a or its progeny were no longer considered regulated articles under the regulations.

II. BACKGROUND

Development of line 19-51a. In a petition dated September 13, 1995 DuPont Company requested a determination from APHIS that line 19-51a, and any progeny derived from it, should no longer be considered regulated articles under APHIS regulations 7 CFR Part 340. Line 19-51a has been considered a regulated article because it was engineered employing a plant pathogenic strain of *Agrobacterium tumefaciens* and its disarmed Ti-plasmid vector. Line 19-51a has no antibiotic resistance gene since the herbicide tolerance conferred by the inserted ALS gene was the basis of selection, and the *ALS* gene, promoter and terminator are all derived from the tobacco plant.

The *ALS* gene in cotton line 19-51a is a chimeric gene derived from two different tobacco ALS genes that both encode herbicide

sensitive versions of ALS. Two resistance mutations were introduced into one of the ALS genes by in vitro site-directed mutagenesis. A DNA fragment containing the resistance mutations was moved into the second ALS gene by using a common restriction enzyme fragment. The gene introduced into cotton line 19-51a, designated chimeric *S4-HrA*, encodes a resistant form of ALS with resistance attributable to two amino acid changes in the protein sequence.

The chimeric *S4-HrA* gene was transformed into *Agrobacterium tumefaciens* strain LBA 4404, a "disarmed" strain of *Agrobacterium* in which the genes responsible for induction of tumors in plants have been deleted from the Ti plasmid. The T-DNA introduced into cotton cultivar Coker 312, other than the ALS gene, contains no other intact prokaryotic or eukaryotic coding sequences.

Polymerase chain reaction (PCR) analyses confirmed that the chimeric *S4-HrA* gene was stably integrated into the cotton genome and transmitted through normal sexual reproduction. Southern blot analyses indicated that two copies of the gene, in tandem repeat, had been introduced at one locus and that DNA beyond the left and right borders had not been introduced into the cotton. Thus the only enzyme expressed by DNA inserted into cotton line 19-51a is the resistant form of ALS enzyme, which confers tolerance to sulfonylurea herbicides and enhances the safety of Staple® herbicide.

The use of cotton plants derived from line 19-51a would enable the farmer to utilize certain sulfonylurea herbicides at a small fraction of an ounce of active ingredient per acre and would increase the safety in use of Staple® herbicide at one to two ounces of active ingredient per acre, especially under more adverse growing conditions. DuPont sulfonylurea herbicides, used alone or in combination with Staple® could be used to provide over-the-top broadleaf weed control, a void in current cotton weed control programs. These herbicides could eventually eliminate or reduce the number of soil applications and present the greatest opportunity to reduce the total amount of herbicide used yearly in United States cotton production. The post emergence activity of these herbicides would allow growers to treat weeds only when needed, which fits in well with conservation (reduced) tillage programs.

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. APHIS' decision on the regulatory status of line 19-51a or its progeny 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 line 19-51a 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 prepared this EA before determining the status of line 19-51a as a regulated article under APHIS regulations. The developer of line 19-51a, DuPont Company, submitted a petition to APHIS requesting that APHIS make a determination that line 19-51a and their progeny shall no longer be considered regulated articles under APHIS regulations (7 CFR Part 340).

This EA was prepared in compliance with: (1) the National Environmental Policy Act of 1969 (NEPA) (42 U.S.C. 4321 et seq.), (2) Regulations of the Council on Environmental Quality for implementing the procedural provisions of NEPA (40 CFR 1500-1508), (3) USDA regulations implementing NEPA (7 CFR Part 1b), and APHIS' NEPA Implementing Procedures (7 CFR part 372; 60 FR 6000-6005, February 1, 1995).

IV. ALTERNATIVES

A. No Action.

Under the Federal "no action" alternative, APHIS would not come to a determination that line 19-51a is no longer a regulated article under the regulations at 7 CFR Part 340. Permits or notifications from APHIS would still be required for introductions of line 19-51a. APHIS might choose this alternative if there were insufficient evidence to demonstrate the lack of plant pest risk from uncontained cultivation of line 19-51a.

B. Determination that line 19-51a is no longer a regulated article.

Under this alternative, line 19-51a 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 line 19-51a. 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 line 19-51a should no longer be considered regulated article. Previous EAs (91-025-02, 91-358-01, and 93-053-01) 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 cultivations, and notifications that 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 line 19-51a.

Additional technical information is included in the determination document appended to this EA, and is incorporated by reference. The determination includes detailed discussions of the biology of cotton, the genetic components used in the construction of line 19-51a and the analyses that lead APHIS to conclude that line 19-51a has no potential to present a plant pest risk.

A. The introduced genes, their products, and the added regulatory sequences controlling their expression do not present a plant pest risk in transformed line 19-51a

Cotton line 19-51a was produced by expressing a mutant *ALS* gene that is insensitive to inhibition by sulfonylurea herbicides. An antibiotic resistance gene was not used as a marker since the herbicide tolerance conferred by the inserted *ALS* gene was the basis of selection. The *ALS* gene, promoter and terminator are all derived from plants. The gene introduced into cotton line 19-51a, designated chimeric *S4-HrA*, encodes a resistant form of ALS with resistance attributable to two amino acid changes in the protein sequence. The chimeric *S4-HrA* gene was constructed by combining fragments of DNA from the *ALS 1* and *ALS 2* genes (Hartnett *et al.* 1990). Mutations analogous to those in the double mutant tobacco line were introduced into the sensitive version of *ALS 2* by site-directed mutagenesis and then a restriction fragment containing the two introduced mutations was subcloned into the sensitive version of the *ALS 1* gene. The two mutations introduced into the *ALS 2* gene result in a proline to alanine substitution at amino acid position 191 and a tryptophan to leucine substitution at amino acid position 568. The chimeric S4-HrA gene is under the control of the ALS 1 gene promoter and 3' terminator. The chimeric *S4-HrA* gene was transformed into *Agrobacterium tumefaciens* strain LBA 4404, a "disarmed" strain of *Agrobacterium* in which the genes responsible for induction of tumors in plants have been deleted from the Ti plasmid. The T-DNA introduced into cotton cultivar Coker 312, other than the *ALS* gene, contains no other intact prokaryotic or eukaryotic coding sequences.

Polymerase chain reaction (PCR) analyses confirmed that the chimeric S4-HrA gene was stably integrated into the cotton genome and transmitted through normal sexual reproduction. Southern blot analyses indicated that two copies of the gene, in tandem repeat, had been introduced at one locus and that DNA beyond the left and right borders had not been introduced into the cotton. Thus the only enzyme expressed by DNA inserted into cotton line 19-51a is the resistant ALS enzyme, which confers tolerance to sulfonylurea herbicides and enhances the safety of Staple® herbicide.

It is clear from our previous EAs and also from the scientific evidence in the published literature that even though live plant pathogenic bacteria were used to develop these transgenic cotton lines, they do not have any inherent ability to cause disease in the transformed plants. As such they do not present a risk of plant pests being introduced into the environment by way of uncontained cultivation of line 19-51a.

B. Potential for line 19-51a 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 because of engineered herbicide resistance. 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 line 19-51a is a line of cotton (*Gossypium hirsutum* L.) known as Coker 312 that exhibits no appreciable weedy characteristics. The *ALS* gene is unlikely to increase weediness of line 19-51a. The sulfonylurea resistance of these plants will confer a selective advantage only when sulfonylurea is applied to the plants. No other attributes of line 19-51a suggest that it be any more "weedy" than traditionally-bred cotton cultivars. Other than the resistance to the herbicide sulfonylurea, line 19-51a has retained the agronomic characteristics of the parental cotton, including the sensitivity to other herbicides.

DuPont has provided data regarding seed germination rates, yield characteristics, disease and pest susceptibilities, compositional analyses, and numerous other tests which support APHIS conclusion that line 19-51a is no more likely to become a weed than cotton developed by traditional breeding techniques.

C. Potential for line 19-51a 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). 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 limited (100-200 feet) (McGregor, 1976).

APHIS considered whether the movement of the chimeric *S4-HrA* gene from line 19-51a 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 sulfonylurea tolerance trait will be inadvertently incorporated into the germplasm of cultivated cotton lines.

D. Potential for line 19-51a to harm other organisms, including agriculturally beneficial organisms and threatened or endangered species.

Consistent with its statutory authority and requirements under NEPA, APHIS evaluated the potential for line 19-51a to directly or indirectly harm other organisms, including those that are recognized as beneficial to agriculture and those that are recognized as threatened or endangered in the United States.

APHIS concluded that the available evidence suggests that line 19-51a 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 sulfonylurea herbicides in the cultivation of line 19-51a, or its offspring will be regulated by the EPA under its existing regulations for the registration of pesticide use. As part of the pesticide registration process, EPA considers the impacts on the environment, including organisms.

E. Potential for line 19-51a to damage agricultural commodities.

APHIS can envision no way in which line 19-51a would damage agricultural commodities. With the exception of the ALS enzyme, the composition and attributes of line 19-51a are indistinguishable from its parental line. There is no indication that the ALS enzyme itself will affect the quality of commodities derived from line 19-51a.

VI. CONCLUSION

APHIS has evaluated the information from the scientific literature as well as information submitted by DuPont that characterized line 19-51a. After careful analysis, APHIS has identified no significant impact to the environment from issuance of a determination that line 19-51a should no longer be a regulated article under APHIS regulations at 7 CFR Part 340. This finding is supported by the following conclusions:

1. Line 19-51a exhibits no plant pathogenic properties. Although a plant pathogen was used in its development, the cotton plants are not infected nor can such plants incite disease in other plants.

2. Line 19-51a is no more likely to become a weed 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. Line 19-51a is unlikely to increase the weediness potential for any other cultivated or wild species with which they can interbreed. The introgression of the *ALS* gene from line 19-51a 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. Line 19-51a will not harm other organisms, including agriculturally beneficial organisms and threatened and endangered species.

5. Line 19-51a should not cause damage to processed agricultural commodities. Seeds of line 19-51a 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 line 19-51a 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 line 19-51a were no longer considered regulated articles under its regulations at 7 CFR Part 340.

VII. LITERATURE CITED

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Response to DuPont Agricultural Products Petition 95-256-01p For a Determination of Nonregulated Status for Sulfonylurea Tolerant Cotton Line 19-51a

Prepared by
United States Department of Agriculture
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I. SUMMARY

In its petition dated September 11, 1995, DuPont Agricultural Products (Dupont) requested a determination from the Animal and Plant Health Inspection Service (APHIS) that sulfonylurea tolerant cotton line 19-51a, and any progeny derived from it, should no longer be considered regulated a article under APHIS regulations 7 CFR Part 340. The sulfonylurea tolerant cotton line 19-51a (hereafter referred to as 19-51a) has been considered a regulated article because it was engineered with DNA sequences derived from the plant pathogen, *Agrobacterium tumefaciens*.

Line 19-51a was developed by recombinant DNA techniques to introduce a sulfonylurea tolerant form of *ALS* gene, which encodes an acetolactate synthase (ALS) enzyme. The ALS enzyme produced in these transgenic cotton lines is a resistant form of the similar enzyme present in all plants, bacteria and fungi, and thereby confers resistance or tolerance to sulfonylurea herbicides. The *ALS* gene used in the cotton line 19-51a is a chimeric gene construct that combines two different *ALS* genes that both encode herbicide sensitive versions of ALS (Mazur *et al.* 1987). Through site directed mutagenesis the tobacco *ALS* genes were altered to confer resistance to sulfonylurea herbicide. This recombinant *ALS* gene designated S4-HrA codes for the resistant from of the ALS enzyme that differs in two amino acids, and confers tolerance to sulfonylurea herbicide in the transgenic cotton line 19-51a. The transgenic cotton line that is the subject of the petition were developed by a widely used technique called Agro-infection which essentially involves using a plant pathogenic strain of *A. tumefaciens*, and its disarmed plasmid vector (Hoekema *et al.* 1983; Ooms *et al.* 1981; Ooms *et al.* 1982). The chimeric S4-HrA gene was cloned into *A. tumefaciens* LBA4404. The S4-HrA gene was transformed into cotton plant cells via a "disarmed" Ti-plasmid vector. The variety of cotton that was used in developing the line 19-51a is the cultivar Coker 312.

Sulfonylurea, the active ingredient of Staple® herbicide, is used as over the top broadleaf weed control in cotton fields. It is claimed that by cultivating cotton line 19-51a, one could use a small fraction of an ounce per acre of the active ingredient of Staple® herbicide. The sulfonylureas are a group of compounds that inhibit ALS, the enzyme that catalyzes the first step in the biosynthesis of the essential amino acids isoleucine, leucine, and valine (LaRossa and Schloss, 1984; LaRossa and Falco, 1984). These compounds inhibit plant growth by inactivating a key enzyme in the essential amino acid biosynthetic pathway. Essential amino acids are not produced by mammals, and they therefore lack the target enzyme, thus contributing to the low toxicity of these herbicides in mammals.

Based on a review of available scientific information, and the data submitted by the applicant in its petition, APHIS has determined that cotton line 19-51a, and its progeny 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 line 19-51a or its progeny.

This determination has been made based on an analysis that revealed that line 19-51a: (1) exhibits no plant pathogenic properties; (2) is no more likely to become a weed than cotton 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 it 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) does 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 19-51a will exhibit new plant pest properties, i.e., properties substantially different from any observed for the cotton line 19-51a 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. Line 19-51a has been considered a "regulated article" under Part 340 of the regulations because it has been engineered with vector DNA sequences derived from the plant pathogenic bacterium, *A. tumefaciens* LBA4404.

Section 340.6 of the regulations, entitled "Petition Process for Determination of Nonregulated Status," provides that a person may petition the Agency to evaluate submitted information and determine that a particular regulated article does not present a plant pest risk and should no longer be regulated. If APHIS determines that the regulated article is unlikely to pose a greater plant pest risk than the unmodified organism, the Agency can grant the petition in whole or in part. As a consequence of such a determination, APHIS permits would no longer be required for field testing, importation, or interstate movement of that article or its progeny.

APHIS decision on the regulatory status of line 19-51a under the regulations in 7 CFR 340, does not release these cotton plants and their progeny from Environment Protection Agency (EPA) and Food and Drug Administration (FDA) regulatory oversight. The regulation of herbicide use on these cotton lines rests with the authority of EPA.

III. PUBLIC COMMENTS

APHIS received one comment each from the Arizona Department of Agriculture and the Texas Department of Agriculture. Both the comments were supportive of the petition for approval.

IV. ANALYSIS OF Line 19-51a

Biology of Cotton: The cotton variety used for developing the line 19-51a is the commercial cotton variety known as Coker 312. 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). 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, 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 *ALS* gene from the lines 19-51a 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 sulfonylurea tolerance trait will be inadvertently incorporated into the germplasm of cultivated cotton lines.

Rationale for Development of Sulfonylurea tolerant Cotton:

Sulfonylurea is the active ingredient of Staple® herbicide is used as over the top broadleaf weed control in cotton fields. It is claimed that by cultivating cotton line 19-51a, one could use a small fraction of an ounce of the active ingredient of Staple herbicide. The sulfonylureas are a group of compounds that inhibit ALS, the enzyme that catalyzes the first step in the biosynthesis of the essential amino acids isoleucine, leucine, and valine

(LaRossa and Schloss, 1984; LaRossa and Falco, 1984). These compounds inhibit plant growth by inactivating a key enzyme in the essential amino acid biosynthetic pathway. Essential amino acids are not produced by mammals, and they therefore lack the target enzyme, thus contributing to the low toxicity of these herbicides in mammals.

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 herticides 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 Staple® as an effective post emergence herbicide with sulfonylurea tolerant cotton may make it possible to reduce the use of pre-emergence herbicides in cotton production.

Development of Line 19-51a:

Line 19-51a was developed by recombinant DNA techniques to introduce a sulfonylurea tolerant form of *ALS* gene, which encodes the acetolactate synthase (ALS). The ALS enzyme produced in 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 sulfonylurea herbicides. The *ALS* gene used in the cotton line 19-51a is a chimeric gene construct that combines two different *ALS* genes that both encode herbicide sensitive versions of ALS. Through site directed mutagenesis the tobacco *ALS* genes were altered to confer resistance to sulfonylurea herbicide. This recombinant *ALS* gene designated S4-HrA codes for the resistant from of the ALS enzyme that differs in two amino acids, and confers tolerance to sulfonylurea herbicide in the transgenic cotton line 19-51a. The transgenic cotton line that is the subject of the petition were developed by a widely used technique called Agro-infection which essentially involves using a plant pathogenic strain of *A. tumefaciens*, and its disarmed plasmid vector (Hoekema *et al.* 1983; Ooms et al. 1981; Ooms *et al.* 1982). The Chimeric S4-HrA gene was cloned into *A. tumefaciens* LBA4404. S4-HrA gene was transformed into cotton plant cells via a "disarmed" Ti-plasmid vector. The variety of cotton that was used in developing the line 19-51a is the cultivar Coker 312.

Line 19-51a was tested in 35 field trials under either APHIS permits or notifications in Arkansas, Delaware, Georgia, Louisiana, Mississippi, North Carolina, Puerto Rico, South Carolina, Tennessee, and Texas. In 1995, fifty field tests have been conducted in 14 states.

Construction of the Binary Vectors Used in Transformation:

A binary vector system based on *A. tumefaciens* plant transformation system was used to transfer the chimeric gene *S4-HrA* into cotton (Bevan, 1984). The vector molecules have been deleted of its tumor causing genes, and the binary vector system consists of two plasmid molecules that function coordinately to effectively transfer the desired gene(s) into the target plant cell genome without transferring unnecessary or extra DNA sequences.

In this instance, plasmid pMH26 was constructed by cloning the modified *ALS* gene into the binary plasmid pZH1 whose backbone DNA sequences were derived from plasmids indigenous to *A. tumefaciens*, *Escherichia coli*, and *Pseudomonas aeruginosa* (Itoh *et al.* 1984; Sutcliffe, 1979; Yanisch-Peron *et al.* 1985). The binary vectors were introduced into *A. tumefaciens* LBA4404 which was used for the agro-infection technique to transfer the gene into the cotton genome.

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 *A. tumefaciens*, and its disarmed plasmid vector (Hoekema *et al.* 1983; Ooms et al. 1981; Ooms *et al.* 1982). The chimeric S4-HrA gene was cloned into *A. tumefaciens* LBA4404. The S4-HrA gene was transformed into cotton plant cells via a "disarmed" Ti-plasmid vector. The variety of cotton that was used in developing the line 19-51a is the cultivar Coker 312.

A. THE INTRODUCED GENES, THEIR PRODUCTS, AND THE ADDED REGULATORY SEQUENCES CONTROLLING THEIR EXPRESSION DO NOT PRESENT A PLANT PEST RISK IN LINE 19-51a.

Southern blot analyses indicate that the line 19-51a contains 2 copies of the *ALS* gene in one or two loci. Once inserted into the chromosome of the cotton plant, the insertion was confirmed to be stably integrated, and that the introduced *ALS* gene is maintained and transmitted in the same manner as any other genes following the Mendelian laws of inheritance. Southern blot analysis also confirmed that no DNA sequences outside the border regions of the T-DNA was introduced into the transformed cotton line 19-51a.

Using a method described by Chaleff and Mauvais (1984), the ALS activity was assayed from the leaves of 19-51a. In this assay, acetolactate, the reaction product of ALS was converted into acetoin by acid quench which in turn is converted into α-naphthol, to a chromogen which absorbs light at a wavelength of 530 nm. The data shows an increased ALS activity no more than two-fold in line 19-51a.

B. Line 19-51a HAS NO SIGNIFICANT POTENTIAL TO BECOME A SUCCESSFUL WEED.

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 line 19-51a is a line of cotton (*Gossypium hirsutum* L.) known as Coker 312 that exhibits no appreciable weedy characteristics. The *ALS* gene is unlikely to increase weediness of line 19-51a. The sulfonylurea tolerance of these plants will confer a selective advantage only when sulfonylurea is applied to the plants. No other attributes of line 19-51a suggest that it be any more "weedy" than traditionally-bred cotton cultivars. Other than the resistance to sulfonylurea herbicide, line 19-51a has retained the agronomic characteristics of the parental cotton, including the sensitivity to other herbicides.

DuPont Agricultural Products Company has provided data regarding seed germination rates, yield characteristics, disease and pest susceptibilities, composition analyses, and numerous other tests which support APHIS' conclusion that line 19-51a is no more likely to become weeds than cotton developed by traditional breeding techniques. Cotton is not considered a weed.

C. Line 19-51a 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* and *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. 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 *ALS* gene from line 19-51a 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 sulfonylurea tolerance trait will be inadvertently incorporated into the germplasm of cultivated cotton lines.

D. Line 19-51a WILL NOT HARM ORGANISMS BENEFICIAL TO AGRICULTURE OR ORGANISMS THAT ARE DESIGNATED AS THREATENED OR ENDANGERED.

APHIS evaluated the potential for the line 19-51a plants to harm organisms either directly or indirectly, particularly those organisms that are recognized as beneficial to agriculture. There is no reason to believe that the cultivation of line 19-51a cotton or its progeny will exert any deleterious effects on organisms recognized as beneficial to agriculture. Likewise, cultivation of line 19-51a will not harm any species designated as threatened or endangered. Line 19-51a produce one enzyme, the herbicide resistant form of ALS, 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 the line 19-51a would be injurious or pathogenic to beneficial organisms such as bees and earthworms.

The definition of line 19-51a encompasses not only the cotton lines that already have been field tested, but also new cotton lines produced through conventional breeding using line 19-51a as one or both parents. APHIS believes that the analysis applied to line 19-51a plants already field tested will apply equally well to these new cotton lines, and that the data provided by DuPont Agricultural Products Company justify the conclusion that such new lines derived from line 19-51a will not present a plant pest risk. The variation in agronomic characteristics among the 19-51a 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 line 19-51a will possess plant pest properties.

E. LINE 19-51a SHOULD NOT CAUSE DAMAGE TO PROCESSED AGRICULTURAL COMMODITIES.

The characteristics of line 19-51a 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, line 19-51a exhibited the typical agronomic characteristics of the parent cotton. In the opinion of APHIS, the components and processing characteristics of line 19-15A 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 cotton line 19-15A developed by DuPont Agricultural Products Company that previously have been field tested under permits and notifications 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 line 19-51a 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) Line 19-51a exhibits no plant pathogenic properties;

2) Line 19-51a is no more likely to become a weed than cotton developed by traditional breeding techniques;

3) Line 19-51a is unlikely to increase the weediness potential for any other cultivated or wild species with which it can interbreed;

4) Line 19-51a will not harm other organisms, including agriculturally beneficial organisms and threatened and endangered species; and

5) Line 19-51a should not cause damage to processed agricultural commodities.

APHIS has also concluded that there is a reasonable certainty that line 19-51a or varieties bred from this line will not exhibit new plant pest properties, i.e., properties substantially different from any observed for line 19-51a plants already field tested, or those observed for cotton in traditional breeding programs.

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