[Docket No. 94–139–2]

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 genetically engineered, insect-resistant cotton lines designated as 531, 757, and 1076 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: June 22, 1995.

ADDRESSES: The determination, an environmental assessment and finding of no significant impact, the petition, and all written comments received regarding the petition may be inspected at USDA, room 1141, South Building, 14th Street and Independence Avenue SW., Washington, DC, between 8 a.m. and 4:30 p.m., Monday through Friday, except holidays. Persons wishing to inspect those documents are asked to call in advance of visiting at (202) 690–2817.

FOR FURTHER INFORMATION CONTACT: Dr. Keith Reding, Biotechnologist, Biotechnology Permits, BBEP, APHIS, 4700 River Road Unit 147, Riverdale, MD 20737–1237; (301) 734–7612. To obtain a copy of the determination or the environmental assessment and finding of no significant impact, contact Ms. Kay Peterson at (301) 734–7612.

SUPPLEMENTARY INFORMATION:

Background

On November 4, 1994, the Animal and Plant Health Inspection Service (APHIS) received a petition (APHIS Petition No. 94–308–01p) from the Monsanto Company (Monsanto) of St. Louis, MO, seeking a determination that cotton lines designated as 531, 757, and 1076 that have been genetically engineered for insect resistance do not present a plant pest risk and, therefore, are not regulated articles under APHIS' regulations in 7 CFR part 340.

On February 9, 1995, APHIS published a notice in the Federal Register (60 FR 7746–7747, Docket No. 94–139–1) 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 April 10, 1995.

APHIS received 69 comments on the Monsanto petition, from cotton farmers, individuals, universities, agricultural experiment stations, cooperative extension service offices, a bank, a chemical company, a cotton researcher, a cotton cooperative association, a gas and oil supplier, and a worker's compensation trust. Sixty-eight commenters either provided information supporting nonregulated status for the subject cotton lines or urged expedited approval to allow commercial planting of the insect-resistant cotton. One commenter cited several issues for further consideration, without recommending approval or denial of the petition. APHIS has provided a summary and discussion of the comments in the determination document, which is available upon request from the individual listed under **FOR FURTHER INFORMATION CONTACT.**

Analysis

Monsanto's cotton lines 531, 757, and 1076 have been genetically engineered to express an insect control protein encoded by the *cryIA(c)* gene that occurs naturally in *Bacillus thuringiensis* subsp. *kurstaki (Btk)*, a common soil bacterium. This protein is effective against such lepidopteran insect pests as cotton bollworm, tobacco budworm, and pink bollworm, and is expressed at a consistent level in the cotton plant throughout the growing season. The subject cotton lines also contain the *nptII* gene which encodes the enzyme neomycin phosphotransferase II. Presence of the NPTII protein confers tolerance to the antibiotic kanamycin and allows selection of the transformed cells in the presence of kanamycin. These genes were stably transferred into the genome of cotton plants using *Agrobacterium tumefaciens*-mediated transformation.

The subject cotton lines have been considered regulated articles under APHIS' regulations in 7 CFR part 340 because they contain gene sequences (vectors, promoters, and terminators) derived from plant-pathogenic sources. However, evaluation of field data reports from field tests of the subject cotton lines conducted since 1992 under APHIS permits or notifications 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 531, 757, and 1076: (1) Exhibit no plant pathogenic properties; (2) are no more likely to become weeds than their nonengineered parental varieties; (3) are not likely to increase the weediness potential of any other cultivated plant or native wild species with which they can interbreed; (4) will not cause damage to raw or processed agricultural

commodities; (5) and are not likely to harm other organisms, such as bees, that are beneficial to agriculture. APHIS has also concluded that there is a reasonable certainty that new lepidopteran-resistant cotton varieties bred from these lines will not exhibit new plant pest properties, i.e., properties substantially different from any observed for the lepidopteran-resistant cotton lines already field tested or those observed for cotton in traditional breeding programs.

The effect of this determination is that insect-resistant cotton lines designated as 531, 757, and 1076 are no longer considered regulated articles under APHIS' regulations in 7 CFR part 340. Therefore, the permit and notification requirements pertaining to regulated articles under those regulations no longer apply to the field testing, importation, or interstate movement of the subject cotton lines 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 the subject cotton lines and lines developed from them are no longer regulated articles under its regulations in 7 CFR part 340. Copies of the EA and the FONSI are available upon request from the individual listed under **FOR FURTHER INFORMATION CONTACT.**

Done in Washington, DC, this 6th day of July 1995.

Terry L. Medley,

Acting Administrator, Animal and Plant Health Inspection Service.

[FR Doc. 95–17080 Filed 7–12–95; 8:45 am]

BILLING CODE 3410–34–P

USDA/APHIS Determination on a Petition 94-308-01p of Monsanto Agricultural Company Seeking Nonregulated Status of Lepidopteran-Resistant Cotton Lines 531, 757, 1076

Environmental Assessment And
Finding of No Significant Impact

June 1995

The Animal and Plant Health Inspection Service (APHIS), United States Department of Agriculture (USDA) has prepared an environmental assessment prior to the determination that lepidopteran-resistant cotton lines 531, 757, and 1076 developed by Monsanto Corporation are no longer deemed regulated articles under 7 CFR 340. This environmental assessment has reached a finding of no significant impact to the environment from its determination that lepidopteran-resistant cotton lines 531, 757, and 1076 or their progeny shall no longer be regulated articles.

John H. Payne, Ph.D.
Acting Director
Biotechnology, Biologics, and Environmental Protection
Animal and Plant Health Inspection Service
United States Department of Agriculture
Date: JUN 22 1995

Key Words: cotton; insect resistance; *Bacillus thuringiensis*; *cryIA(c)*; delta-endotoxin protein; marker gene; *nptII*; neomycin phosphotransferase; kanamycin resistance.

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Environmental Assessment

I. SUMMARY

USDA/APHIS has prepared an Environmental Assessment in response to a petition (APHIS Number 94-308-01p) from Monsanto Agricultural Company, Inc., St. Louis, Missouri (hereafter referred to as Monsanto) for the determination of their nonregulated status of their genetically engineered lines of cotton (*Gossypium hirsutum L.*) designated "Bollgard™ cotton Line 531, 757, and 1076" (hereafter referred to as lepidopteran-resistant cotton). Monsanto requested a determination from APHIS that these genetically engineered cotton lines no longer be considered regulated articles under 7 CFR Part 340. Lepidopteran insect resistant cotton lines are defined as those cotton lines that express *cryIA(c)* gene coding for cryIA(c) toxin protein from the soil bacterium *Bacillus thuringiensis* var. *kurstaki* that confers insect resistance to lepidopteran insects in general, and cotton bollworm, tobacco budworm, and pink bollworm, in particular. They also express the *nptII* gene from *Escherichia coli* coding for the neomycin phosphotransferase enzyme that confers resistance to the antibiotic kanamycin.

Separate Environmental Assessments (EA) were prepared before granting the permits for field trials of these lepidopteran-resistant cotton lines. These EA addressed questions pertinent to plant pest risk issues concerning the conduct of field trials under physical and reproductive confinement, but they do not address several issues that are of relevance to the unconfined growth of genetically engineered cotton lines. With respect to these new issues, APHIS concludes the following:

1. Lepidopteran-resistant cotton lines 531, 737, and 1076 exhibit no plant pathogenic properties. Although pathogenic organisms were used in their development, these cotton plants are not infected nor can they cause disease in other plants.

2. Lepidopteran-resistant cotton lines are no more likely to become weeds than the nonengineered parental varieties. Cotton is not a serious, principal or common weed pest in the U.S., and there is no reason to believe that expression of the lepidopteran-resistane trait would cause these cotton plants to become weed pests.

3. The potential for gene introgression from genetically engineered cotton lines into wild or cultivated sexually compatible plants is very low, and such events are highly unlikely to increase the weediness potential of any resulting progeny.

4. Lepidopteran-resistant cotton lines are substantially equivalent in composition and lint quality to their nontransgenic counterparts and should have no adverse impacts on raw or processed agricultural commodities.

5. Lepidopteran-resistant cotton lines exhibit no significant potential either to harm organisms beneficial to the agricultural

ecosystem or to lead to increased pest nature of other nontarget insect pests.

6. The use of genetically engineered cotton lines should present no greater risk of decreasing the ability to control cotton bollworm, tobacco budworm, and pink bollworm than any other method of insect control in cotton.

Therefore, after a review of the available evidence, APHIS believes that lepidopteran-resistant cotton lines will be just as safe as nontransgenic cotton plants that are typically grown using other methods to control cotton bollworm, tobacco budworm, and pink bollworm, and which are not subject to regulation under 7 CFR Part 340. APHIS concludes that there should be no significant impact on the human environment if these lepidopteran-resistant cotton lines were no longer considered regulated articles under regulations at 7 CFR Part 340.

II. BACKGROUND

A. Development Of Lepidopteran-Resistant Cotton Lines.

Monsanto has submitted a "Petition for Determination of Non-regulated Status" to the USDA, APHIS for three lepidopteran-insect resistant cotton (Bollgard™) lines 531, 757, and 1076 are defined as those cotton lines that express *cryIA(c)* gene coding for cryIA(c) toxin protein from the soil bacterium *Bacillus thuringiensis* var. *kurstaki* that confers insect resistance to lepidopteran-insects in general, and cotton bollworm, tobacco budworm, and pink bollworm, in particular. Upon ingestion of this protein by susceptible insects, feeding is inhibited, eventually resulting in death. The protein coding region of the gene was modified for optimal expression in plants. To express the gene, this region is fused to the promoter derived from the 35S gene of cauliflower mosaic virus (CaMV) with a duplicated enhancer region and to the nontranslated region of the soybean alpha subunit of the beta-conglycinin gene which provides the mRNA polyadenylation signals (7S 3' terminator sequence). The lines also express the *nptII* gene from *Escherichia coli* coding for the neomycin phosphotransferase enzyme, which confers resistance to the antibiotic kanamycin. These two genes were introduced into cotton lines via an *Agrobacterium*-mediated transformation protocol. This is a well-characterized procedure that has been used widely for over a decade for introducing various genes of interest directly into plant genomes.

These lepidopteran-resistant cotton lines have been field tested since 1991 in the major cotton growing regions of the United States under 10 APHIS permits numbers 90-347-01, 91-144-01, 91-347-02, 93-011-02, 93-011-05, 93-056-05, 94-025-01, 94-026-03, 94-027-03, and 94-054-02 in 14 states and 125 sites. Lepidopteran-resistant cotton lines have been evaluated extensively in laboratory and field experiments to confirm that these exhibit the desired agronomic characteristics and

do not present a plant pest risk. Although the field tests have been conducted in agricultural settings, the permit conditions for the tests have stipulated physical and reproductive confinement from other plants.

B. APHIS Regulatory Authority.

APHIS regulations at 7 CFR Part 340, which were promulgated according to the 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. An organism is no longer subject to the regulatory requirements of 7 CFR Part 340 when it is demonstrated not to present a plant pest risk. 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. Lepidopteran-resistant cotton lines described in the Monsanto petition have been considered regulated articles because noncoding DNA regulatory sequences and portions of the plasmid vector are derived from plant pathogens.

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

C. EPA And FDA Regulatory Authority

These lepidopteran-resistant cotton lines are also subject to regulation by other agencies. The EPA is responsible for the regulation of pesticides under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136 *et seq.*). FIFRA requires that all pesticides, including insecticides, be registered prior to distribution or sale, unless exempt by EPA regulation. Accordingly, Monsanto has submitted to EPA an application to register this plant-pesticide, ie. the B.t.k. control protein as produced by the *cryIA(c)* gene and its controlling sequences in these lepidopteran-resistant cotton lines. On February 15, 1994, EPA announced receipt of this application (EPA File Symbol 524-UTI) in the Federal Register (59 FR 49663). This is one of the applications for registration of a transgenic plant pesticide under section 3(c) of FIFRA, as amended, in which a plant has been genetically altered to produce a pesticide.

The EPA has not yet announced its final decision on this registration application; however, the Office of Pesticides Program has made available in the public docket a preliminary scientific position document regarding this registration application in preparation for a FIFRA Scientific Advisory Panel meeting, as announced in the Federal Register, January 25, 1995, Docket No. 95-2009, p. 4910. Before a product may be registered as a pesticide under FIFRA, it must be shown that when used in accordance with widespread and commonly recognized practice, it will not generally cause unreasonable adverse effects on the environment. FIFRA also authorizes EPA to issue Experimental Use Permits (EUP) and otherwise regulate the use of unregistered pesticides under FIFRA section 3(a). EUPs are generally issued (as authorized under FIFRA section 5 and 40 CFR part 172) for large-scale testing of pesticides on more than 10 cumulative acres of land. Contained within the scope of the regulation, however, is the presumption that small-scale testing, i.e., on not more than 10 cumulative acres of land, does not require an EUP provided that the crops are destroyed or an appropriate tolerance is in place (40 CFR 172.3(a)).

Under the Federal Food, Drug, and Cosmetic Act (FFDCA) (21 U.S.C. 301 *et seq.*), pesticides added to (or contained in) raw agricultural commodities generally are considered to be unsafe unless a tolerance or exemption from tolerance has been established. Residue tolerances for pesticides are established by EPA under the FFDCA; and the FDA enforces the tolerances set by the EPA. Monsanto has submitted to the EPA a pesticide petition (PP 3F4273) proposing to amend 40 CFR part 180 to establish a tolerance exemption for residues of the plant pesticide active ingredient B.t.k. the insect control protein as expressed in plant cells. On December 8, 1993, EPA announced receipt of this petition [58 FR 64583-64584]. EPA announced its decision on this petition in which the agency approved a limited registration to produce large quantities of seeds (March 30, 1995). The EPA has announced a final rule establishing an exemption from the requirement of a tolerance for residues of *nptII* and the genetic material necessary for its production when used as a plant pesticide inert ingredient (59 FR 49351-49353, Docket No. 94-23762) as it is considered in the lepidopteran-insect resistant cotton lines.

Safety concerns for human and animal consumption of products with kanamycin resistance are also specifically addressed by the FDA in 21 CFR Parts 173 and 573. The FDA policy statement concerning regulation of products derived from new plant varieties was published in the Federal Register on May 29, 1992, and appears at 57 FR 22984-23005. The Monsanto Company has satisfactorily completed their food safety and nutritional assessment as recommended under this FDA policy statement.

III. PURPOSE AND NEED

Monsanto, submitted this petition to USDA, APHIS requesting that APHIS make a determination that lepidopteran-resistant cotton lines shall no longer be considered regulated articles under 7 CFR Part 340. APHIS has prepared this EA to make a determination on the status of lepidopteran-resistant cotton lines as regulated articles under APHIS regulations.

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; 60 FR 6000-6005).

Consistent with the "Coordinated Framework for Regulation of Biotechnology" (51 FR 23302-23350, June 26, 1986), when appropriate, APHIS and the EPA have coordinating their review of these lepidopteran-resistant cotton lines to avoid duplication and assure that all relevant issues are addressed. Therefore, reference is made to EPA review documents which address certain environmental issues.

IV. ALTERNATIVES

A. No Action.

Under the Federal "no action" alternative, APHIS would not come to a determination that lepidopteran-resistant cotton lines are not regulated articles under the regulations at 7 CFR Part 340. Permits or acknowledgement of notifications from APHIS would still be required for introductions of lepidopteran-resistant cotton lines. APHIS might choose this alternative if there were insufficient evidence to demonstrate the lack of plant pest risk from unrestricted cultivation of lepidopteran-resistant cotton lines.

B. Determination That Lepidopteran-Resistant Cotton Lines Are No Longer Regulated Articles APHIS Under Federal Plant Pest Act and Federal Quarantine Act.

Under this alternative, lepidopteran-resistant cotton lines 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 lepidopteran-resistant cotton lines. One basis for this determination could be a "Finding of No Significant Impact" under the National Environmental Policy Act of 1969 (42 USC 4331 *et seq.*; 40 CFR 1500-1509; 7 CFR Part 1b; 60 FR 6000-6005).

V. AFFECTED ENVIRONMENT AND POTENTIAL ENVIRONMENTAL IMPACTS

This EA addresses potential environmental impacts from a determination that lepidopteran-resistant cotton lines should no longer be considered regulated articles under APHIS regulations at 7 CFR Part 340. Previous EA prepared by APHIS for permits to field test lepidopteran-resistant cotton lines have addressed various attributes of lepidopteran-resistant cotton lines. This EA discusses the genetic modification, and the potential environmental impacts that might be associated with the unconfined cultivation of lepidopteran-resistant cotton lines.

Additional technical information is included in the determination document appended to this EA and the EA prepared for contained field tests under APHIS permits, 90-347-01, 91-144-01, 91-347-02, 93-011-02, 93-011-05, 93-056-05, 94-025-01, 94-026-03, 94-027-03, and 94-054-02. The field tests took place at 125 sites in the following 14 states: Alabama, Arizona, Arkansas, California, Florida, Georgia, Hawaii, Louisiana, Mississippi, Missouri, North Carolina, South carolina, Tennessee, and Texas. The EA for all these tests contain detailed discussions of the biology of cotton, the genetic components used in the construction of lepidopteran-resistant cotton lines, and the analyses that lead APHIS to conclude that lepidopteran-resistant cotton lines have no potential to pose plant pest risks.

This EA is concerned with potential environmental impacts from the unrestricted introduction of lepidopteran-insect resistant cotton.

A. Potential For Lepidopteran-Resistant Cotton Lines To Exhibit Increased Weediness Relative To Traditionally Bred Cotton Varieties

Almost all definitions of weediness stress as core attributes the undesirable nature of weeds from the point of view of humans; from this core, individual definitions differ in approach and emphasis (Baker, 1965; de Wet and Harlan, 1975; Muenscher, 1980). In further analysis of weediness, Baker (1965) listed 12 common weed attributes, almost all pertaining to sexual and asexual reproduction, which can be used as an imperfect guide to the likelihood that a plant will behave as a weed. Keeler (1989) and Tiedje et al. (1989) have adapted and analyzed Baker's list to develop admittedly imperfect guides to the weediness potential of transgenic plants; both authors emphasize the importance of looking at the parent plant and the nature of the specific genetic changes.

The parent plant in this petition, *G. hirsutum*, does not show any appreciable weedy characteristics. The genus also seems to be devoid of any such characteristics; although some New World cottons show tendencies to "weediness" (Fryxell, 1979; Haselwood et al., 1983), the genus shows no particular weedy aggressive tendencies. The standard texts and list of weeds give no indication that cotton is clearly regarded as a weed anywhere (Holm et al., 1979; Muenscher, 1980; Reed, 1970; Weed Science Society of America, 1989). Any reports that

cottons behave as a weed are rare and anecdotal, and vague as to the nature of the problem.

The single trait introduced into cotton (lepidopteran-resistance) is unlikely to increase weediness of this cotton. Weediness is a multigenic trait. Assuming that domesticated cotton has the potential to become a weed, any increase in the weediness of the transgenic cotton plant would have to result from the transgenic plant having a competitive advantage over the parental, nontransgenic cotton line (Tiedje et al., 1989; Office of Technology Assessment, 1988). Because lepidopteran-resistant cotton is expected to be cultivated like any other cotton in a managed agricultural ecosystem, the likelihood that sufficient selective pressure would be present for the lepidopteran-resistant cotton to become a weed is low.

No other variation seen in lepidopteran-resistant cotton lines is indicative of increased weediness. Monsanto data from greenhouse studies show a variation in germination rates among transgenic seed lines but no evidence of specific changes in the rate from parent to transgenic plant compared to nontransgenic cottons was found. In addition, Monsanto field reports show no obvious increase in volunteers from seed, regrowth from stubble, or increase in seed dormancy. Lint characteristics showed no changes from that of nontransgenic cotton. Monsanto's report on lint characteristics showed practically no changes from those of nontransgenic cotton. Monsanto did observe some decrease in boll size in line 531, the overall yield of line 531 cotton was superior to that of nontransgenic cotton. Even if such a decrease is reproducible on a larger scale, APHIS believes that no competitive advantage affecting weediness would be conferred on the transgenic plants by this change. APHIS believes that a relationship between seed size and increase in weediness potential should only apply in small-seeded crops, in which seed dispersal is affected by factors like wind, and not in large-seeded crops like cotton.

With respect to potential effects of the cultivation of the lepidopteran-resistant cotton outside the United States, APHIS notes that there is already considerable cultivation of nontransgenic cotton around most centers of cotton diversity. The major threat to many relatives of cotton appears to be habitat destruction (Fryxell, 1979). Several other factors are relevant. (1) Crop plants and seeds exported from the United States, whether transgenic or non-transgenic varieties, are still subject to the phytosanitary restrictions of the importing nation. (2) APHIS has no jurisdiction over agricultural practices in foreign nations and our action does not constitute approval for field testing or commercialization of this cotton in any other nation. (3) Foreign laws restricting or regulating field testing or commerce with transgenic cotton are unaffected by our action. (4) APHIS has no jurisdiction over approval for the use of lepidopteran-resistant cotton plants in foreign nations.

Scenarios in which an impact of lepidopteran-resistant cotton on wild cotton varieties is envisioned depend, at a minimum, on a biologically unlikely scenario coupled with a failure of regulatory oversight in a foreign nation. Knowledge about the ecology of wild cottons and the insects with which they interact is incomplete. APHIS believes that the lack of increased weediness observed for the transgenic cotton lines strongly suggests that any potential gene transfer to wild cotton would not likely result in increased weediness on their part.

B. Potential Impacts Associated With Potential Gene Introgression From Lepidopteran-resistant Cotton Lines To Sexually Compatible Plants (With Wild and Cultivated Relatives)

None of the relatives of cotton found in the United States (*G. barbadense*, *G. thurberi*, and *G. tomentosum*) shows any weedy tendencies. Successful sexual transmission of genetic material via pollen is possible only to certain cotton relatives. In the United States, the compatible species are *G. hirsutum* (wild or under cultivation), *G. barbadense* (cultivated Pima cotton), and *G. tomentosum*.

Lepidopteran-resistant cotton is chromosomally compatible with wild *G. hirsutum*. However, according to Dr. Paul Fryxell of Texas A & M University (personal communication), a leading authority on the systematics and distribution of these species, wild cottons are found only in southern Florida (virtually exclusively in the Florida Keys), whereas cultivated cottons are found in northernmost portions of the State. Other wild *G. hirsutum* is to be found along the Mexican coast, largely along the Yucatan, and populations do not extend as far north as the Texas border. *G. hirsutum* has also been grown in several United States Territories and Possessions, and may even to a greater or lesser degree be spontaneous or naturalized in places such as the Northern Mariana Islands, Puerto Rico, and the Virgin Islands. However, there are no peculiarities of cotton in these areas that would require unique review. Most wild *G. hirsutum* populations are geographically isolated from cultivated cotton, and do no cross with native or cultivated cotton species (Alan C. York, North Carolina State University, personal communication). Even if the non-agricultural land containing any wild cotton populations were near sites of commercial cotton production, there would be no significant impacts, APHIS believes, because: (1) any potential effects of the trait are not expected to alter the weediness of the wild cotton; and (2) wild cotton populations have not been actively protected, but have in fact been, in some locations such as Florida, subject in the past to Federal eradication campaigns, because they can serve as potential hosts for the boll weevil, *Anthonomus grandis* Boh.

Gossypium thurberi, the wild relative found in Arizona, is not compatible with pollen from *G. hirsutum*, so that genetically engineered insect resistant cotton can have no effect on this species. Movement to *G. hirsutum* and *G. barbadense* is possible if suitable

insect pollinators are present, and if there is a short distance from transgenic plants to recipient plants. Any physical barriers, intermediate pollinator-attractive plants, and other temporal or biological impediments would reduce the potential for pollen movement.

Movement of genetic material from cultivated cottons varieties that have been engineered for lepidopteran resistance to *G. tomentosum* is more speculative (see determination for discussion). The wild species is chromosomally compatible with *G. hirsutum*, but there is uncertainty about the possibility for pollination. The flowers of *G. tomentosum* seem to be pollinated by moths, not bees, and they are reportedly receptive at night, not in the day. Both these factors greatly lessen the possibility of cross-pollination. There have been reports based on morphological suggestions (Stephens, 1964; Fryxell, 1979) that *G. tomentosum* may be losing its genetic identity from hybridization with cultivated cottons by unknown means. However, the most recent data, from DeJoode and Wendel (1992), indicate that despite the morphological suggestion of such hybrid populations, biochemical (allozyme) studies show no evidence of any such changes. Major factors influencing the survival of *G. tomentosum* are construction and urbanization, i.e., habitat destruction (Fryxell, 1979). APHIS believes that it is these factors, rather than gene movement from cultivated cottons, that are of real significance to this species. Cotton lines bred by traditional means, which should be no more or less likely to interbreed with *G. tomentosum* than genetically engineered insect resistant cotton, are not considered to pose a threat to the wild cotton and are not subject to particular State or Federal regulation on this basis. Neither the weediness nor the survival of *G. tomentosum* will be affected by the cultivation of genetically engineered insect resistant cotton because: the transgenic variety poses no increased weediness itself; the two species are unlikely to successfully cross in nature; and the added traits will confer no selective advantage in the wild species habitat.

In contrast to the situation with *G. tomentosum*, gene movement from *G. hirsutum* to *G. barbadense* is widespread in advanced cultivated stocks. However, it is conspicuously low or absent in material derived from natural crosses such as that from Central America or the Caribbean where *G. hirsutum* and *G. tomentosum* grow together. The absence of natural introgression may be caused by any one of several isolating mechanisms of pollination, fertilization, ecology, gene incompatibility, or chromosome incompatibility (Percy and Wendel, 1990). Movement of gene material from genetically engineered insect resistant cotton to cultivated or occasional non-cultivated *G. barbadense* would therefore not likely occur at a high level. Any movement of genetic material from genetically engineered insect resistant cotton cottons into *G. barbadense* is likely to be the result of intentional breeding practice rather than accidental crossing. Even if such movement did occur, it would not offer the progeny any clear selective advantage over the parents use.

C. Potential Impact On Nontarget Organisms Including Beneficial Organisms Such As Bees And Earthworms

Consistent with its statutory authority and requirements under NEPA, APHIS evaluated the potential for the lepidopteran-resistant cotton plants and plant products and the *Btk* insect control protein to have damaging or toxic effects directly or indirectly on nontarget organisms, particularly those that are recognized as beneficial to agriculture and to those which are recognized as threatened or endangered in the United States. APHIS also considered potential impacts on other "nontarget" pests, since such impacts could have an impact on the potential for changes in agricultural practices.

CryIA(c), expressed in lepidopteran-resistant cotton lines, shows a strict host-range specificity for lepidopteran insects and has no deleterious effects on nontarget organisms. Invertebrates such as earthworms, and all vertebrate organisms, including non-target birds, mammals and humans, are not expected to be affected by the *Btk* insect control protein because they would not be expected to contain the receptor protein found in the midgut of target insects. Results from high dose feeding studies on bobwhite quail, rats, non-lepidopteran insects, birds, mammals and mice demonstrated no adverse effects. Ecological effect studies submitted to the EPA in support of the earlier registration of foliar microbial *Btk* pesticides indicated no unreasonable adverse effects on nontarget insects, birds, and mammals (EPA, 1988).

There is no reason to believe that deleterious effects or significant impacts on nontarget organisms, including beneficial organisms, would result from the cultivation of lepidopteran-resistantinsect resistant cotton. The novel proteins that will be expressed in lepidopteran resistant cotton are not known to have any toxic properties on any nontarget organisms. The lack of known toxicity for these proteins and the low levels of expression in plant tissue suggest no potential for deleterious effects on beneficial organisms such as bees and earthworms. APHIS has not identified any other potential mechanisms for deleterious effects on beneficial organisms. In addition, there is no reason to believe that the presence of lepidopteran-resistant cotton would have any effect on any other threatened or endangered species in the United States. There is no evidence of any endangered or threatened species of lepidopteran insects feeding on cotton, and as such, no effects of the CryIA(c) protein on them are predicted. There is no reason to believe that deleterious effects or significant impacts on nontarget organisms, including beneficial organisms, would result from the NPTII protein conferring kanamycin resistance used as a selectable marker during development of transgenic cotton lines.

D. Potential Impacts On Agricultural Practices Associated With The Cultivation Of Lepidopteran-resistant Cotton Plants And The Development of Insect Resistance To The *Btk* Insect Control Protein

APHIS considered the potential impacts associated with the cultivation of lepidopteran-resistant cotton plants on current agricultural practices used to control lepidopteran insects in general, and cotton bollworm, tobacco budworm, and pink bollworm, in particular. Pages 100-105 of the Monsanto petition discuss the impact of the lepidopteran-resistant cotton lines on cotton pest management. Monsanto strategy for maximizing the utility of these plants and delaying the development of insects resistant to the CryIA(c) insect control protein is outlined in the Determination. Their strategy was also submitted to the EPA in support of the registration of the CryIIIA and CryIA(c) proteins expressed in plants as a plant-pesticide. Since this evaluation has been made available by the EPA for the Scientific Advisory Panel meeting (Matten, EPA, 1994), the details will not be presented by APHIS. The development of effective resistance management strategies is an ongoing process, and APHIS will offer comments and suggestions, as appropriate, to the EPA and Monsanto to assist in this process. The EPA has stated that they are committed to working with Monsanto to develop product labels and informational brochures that include instructions on the proper use of the lepidopteran-resistant cotton lines consistent with resistance management. For a full discussion of current agricultural practices used to control lepidopteran insect pests of cotton and the impact of lepidopteran-resistantinsect resistant cotton lines, see both the Response to Comments and Section IV.F. of the Determination.

Both larvae and adult lepidopteran insects cause severe damage to cotton crops, and cultivation of cotton consumes the largest amount of chemical insecticides of any crop cultivated worldwide. Cultural control methods, biological and conventional insecticides, and biological control agents currently used or being developed for control of insect pests in cotton are discussed in more detail in the Determination. There is no doubt that cultivation of the lepidopteran-resistant cotton plants could reduce the use of chemical pesticides, some of which are becoming obsolete because of insect resistance (see petition pages 14-16).

There are currently no commercially available cotton cultivars that are resistant to lepidopteran insects. If commercialized, the transgenic insect resistant cotton plants could offer an important alternative to chemical insecticides, particularly to those for which resistance has already developed. They will also offer a more flexible, effective alternative for season-long control of lepidopteran insects compared to the use of some foliar microbial products. By the same token, widespread and inappropriate use of either lepidopteran-resistant cotton or foliar microbial products can and will most likely accelerate the appearance of lepidopteran populations resistant to the *Btk* insect control protein. Calculating

the rate at which resistance will develop using either approach is difficult to predict because it depends on several factors: (1) the resistance management strategies and their acceptance and effective implementation by growers; (2) the genetics of lepidopteran resistance to this insecticide; and (3) the population and behavioral biology of the lepidopteran insect pests (Roush, 1994; Tabashnik, 1994a and b; Gould et al., 1994). The lack of field-selected resistant lepidopteran insect populations precludes the direct testing of the validity of models to predict the rate with which lepidopteran insects will develop resistance using different management strategies.

The implementation of an active resistance management plan that is scientifically sound and acceptable to growers should delay the onset of resistance and provide alternative strategies and methods for managing or containing resistant populations if and when they occur. For example, it may be possible to control resistant lepidopteran insect populations by the use of alternative cultural control practices and alternate insecticides, particularly those to which lepidopteran insects have not yet been exposed. If resistant populations persist, insecticides based on the CryIA(c) protein would no longer be effective for controlling lepidopteran pests on cotton or on other crops for which these insecticides are registered.

APHIS has concluded that there is unlikely to be any significant adverse impact on agricultural practices associated with the appropriate use of lepidopteran-resistant cotton plants. This analysis is based on a consideration of (1) the geographical locations where the lepidopteran-resistant cotton plants will be grown, (2) the major production areas for cotton plants that are subject to lepidopteran pests pressure, (3) the usage of insecticides on these crops, and 4) the availability of alternative lepidopteran pest control measures. Resistance development in insect pest populations is a risk associated with the deployment of insecticides. But in this respect, cultivation of lepidopteran-resistant cotton plants should have no greater impacts on the control of lepidopteran pests in cotton plants and other crops than the widely practiced method of applying insecticides to control lepidopteran insect pests on cotton cultivars. Monsanto has stated that it is in their best interest to delay resistance. The EPA has stated that they will work with Monsanto to develop product labels and informational brochures that are consistent with resistance management, and this should help define the appropriate use of these cotton plants. Should resistant lepidopteran insect populations evolve, it may be possible to limit the persistence and spread of resistant populations. But as with conventional insecticides, where resistance develops, growers will lose the capability to use particular *Btk* insecticides to control lepidoptera on cotton. Although Btk formulations may also be a registered for use on corn, okra, soybeans and tomatoes, occurance of CryIA(c)-resistant lepidopteran insects in cotton fields should have minimal impacts on *Btk* uses on these crops. The only potential for any impact would occur whem the crops are cultivated very near, or in rotation with, the lepidopteran-resistant cotton. Since these

insecticides are currently used infrequently in the major areas of production for these crops, and other options exist for the control of lepidopteran pests, the impact should be minimal.

VI. CONCLUSIONS

APHIS has evaluated information from the scientific literature and data submitted by Monsanto regarding characterized lepidopteran-resistant cotton lines. After careful analysis, APHIS has identified no significant impact to the environment from issuance of a determination that lepidopteran-resistant cotton lines should no longer be regulated articles under APHIS regulations at 7 CFR Part 340.

APHIS has considered the foreseeable consequences of issuing a determination that these lepidopteran-resistant cotton lines are no longer regulated articles, and has reached the following conclusions:

1. Lepidopteran-resistant cotton lines 531, 737, and 1076 exhibit no plant pathogenic properties. Although pathogenic organisms were used in their development, these cotton plants are not infected nor can they incite disease in other plants.

2. Lepidopteran-resistant cotton lines are no more likely to become weeds than insect-resistant cotton plants developed by traditional breeding techniques. Cotton is not a serious, principal or common weed pest in the U.S., and there is no reason to believe that ecpression of the insect resistant trait would cause these cotton plants to become weed pests.

3. The potential for gene introgression from lepidopteran-resistant cotton lines into wild or cultivated sexually compatible plants is very low, and such events are highly unlikely to increase the weediness potential of any resulting progeny.

4. Lepidopteran-resistant cotton lines are substantially equivalent in composition and lint quality to their nontransgenic counterparts and should have no adverse impacts on raw or processed agricultural commodities.

5. Lepidopteran-resistant cotton lines exhibit no significant potential either to harm organisms beneficial to the agricultural ecosystem or to lead to increased pest nature of other nontarget insect pests.

6. The responsible use of lepidopteran-resistant cotton lines should present no greater risk of decreasing the ability to control cotton bollworm, tobacco budworm, and pink bollworm than any other method of insect control in cotton.

Therefore, after review of the available evidence, APHIS concludes that lepidopteran-resistant cotton lines will be just as safe to grow

as any other nontransgenic 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 lepidopteran-resistant cotton lines were no longer considered regulated articles under its regulations (7 CFR Part 340).

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RESPONSE TO MONSANTO PETITION 94-308-01p FOR DETERMINATION OF NONREGULATED STATUS FOR LEPIDOPTERAN-RESISTANT COTTON LINES

June 1995

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

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I. Executive Summary

This Animal and Plant Health Inspection Service determination has been made in response to a petition received on November 4, 1994, from Monsanto Company of St. Louis, Missouri. The petition seeks a determination from APHIS that Bollgard™ cotton does not present a plant pest risk and is therefore no longer a regulated article. On February 9, 1995, APHIS announced receipt of the Monsanto petition in the Federal Register (60 FR 7746-7747) and stated that the petition was available for public view. APHIS also indicated its role, as well as those of the Environmental Protection Agency (EPA) and the Food and Drug Administration (FDA), in regulation of lepidopteran-resistant cotton, its food products, and the pesticide it produces. APHIS invited written comments from the public to be submitted on or before April 10, 1995 on whether lepidopteran-resistant cotton poses a plant pest risk.

Based on a review of scientific data, APHIS has determined lepidopteran-resistant cotton lines do not present a plant pest risk and are therefore no longer regulated articles under its regulations at 7 CFR Part 340.

Bollgard™ cotton, as defined by its developer (Monsanto Company, St. Louis, Missouri), is a cotton cultivar or progeny of a cotton line containing the *cryIA(c)* gene (a δ-endotoxin gene from *Bacillus thuringiensis* subsp. *kurstaki*). Lepidopteran-resistant cotton may also contain: *nptII*, from the bacterium *Escherichia coli* which encodes the enzyme aminoglycoside 3'-phosphotransferase II for resistance to the antibiotic kanamycin; *aad*, from the transposon Tn7 which encodes the enzyme aminoglycoside adenylyltransferase for bacterial resistance to the antibiotics spectinomycin and streptomycin; associated DNA sequences that regulate the expression of the gene products; the *oriV* origin of replication from the broad host range plasmid RK2; and the T-DNA border sequence from the *Agrobacterium tumefaciens* Ti plasmid. Expression of the *cryIA(c)* and *nptII* genes is regulated by the 35S cauliflower mosaic virus promoter with or without the duplicative enhancer region (Kay et al., 1987) or an alternative promoter, claimed as Confidential Business Information. Expression of the genes is terminated using either the 7S 3' sequence of the soybean alpha subunit of the beta-conglycinin gene or the *nos* 3' nontranslated region of the nopaline synthase gene. Each of these sequences is discussed in detail in section IV of this determination.

APHIS regulations at 7 CFR Part 340, which were promulgated under the 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, regulate the introduction (importation, interstate movement, or release into the environment) of certain genetically engineered organisms and products. An organism is not subject to the regulatory requirements of 7 Part 340 when it is demonstrated not to present a plant pest risk. Section 340.6 of the regulations, entitled "Petition Process for Determination of

Nonregulated Status", provides that a person may petition the Agency to evaluate submitted data and determine that a particular regulated article does not present a plant pest risk and should no longer be regulated. If the agency determines that the regulated article does not present a risk of introduction or dissemination of a plant pest, the petition would be granted. This allows for unregulated introduction of the regulated article in question.

The lepidopteran-resistant cotton has been field tested under 11 APHIS permits or notifications (90-347-01, 91-144-01, 91-347-02, 93-011.02, 93-011-05, 93-056-05, 94-025-01, 94-026-03, 94-027-03, and 94-054-02). The field tests were conducted at 125 sites in the following 14 states: Alabama, Arizona, Arkansas, California, Florida, Georgia, Hawaii, Louisiana, Mississippi, Missouri, North Carolina, South Carolina, Tennessee, and Texas. Monsanto, in Part IV of its petition request, has provided field data reports from the completed field trials. All field trials were performed essentially under conditions of reproductive confinement.

Lepidopteran-resistant cotton contains components from organisms that are known plant pathogens, i.e., the bacterium *Agrobacterium tumefaciens* and cauliflower mosaic virus. Lepidopteran-resistant cotton has therefore been a regulated article under APHIS jurisdiction. Field tests in 1990, 1991, 1992, 1993, and 1994 have been conducted in accordance with APHIS regulations. An APHIS determination that lepidopteran-resistant cotton does not present a plant pest risk is based on an analysis of data provided to APHIS by Monsanto and other relevant published scientific data obtained by APHIS concerning the components of lepidopteran-resistant cotton, observable properties of the cotton lines themselves, and the APHIS Environmental Assessments of field tests conducted under permit. From this review, we have determined that these lepidopteran-resistant cotton lines: (1) do not exhibit plant pathogenic properties; (2) are no more likely to become weeds than their nonengineered parental varieties; (3) are not likely to increase the weediness potential for any other cultivated plant or native wild species with which the organisms can interbreed; (4) will not cause damage to processed agricultural commodities; and (5) are not likely to harm other organisms, such as bees and earthworms, that are beneficial to agriculture. In addition, we have determined that there is a reasonable certainty that new progeny lepidopteran-resistant cotton lines bred from these lines will not exhibit new plant pest properties, i.e., properties substantially different from any observed for the lepidopteran-resistant cotton lines already field tested, or those observed for cotton in traditional breeding programs.

Monsanto has provided general information and data from field testing of three lepidopteran cotton lines. Our determination applies to cotton lines that fit the Monsanto definition of lepidopteran-resistant cotton and that have been field tested under permit or notification. The effect of this determination is that such cotton lines will no longer be considered regulated articles under APHIS

regulations at 7 CFR Part 340. Permits under those regulations will no longer be required from APHIS for field testing, importation, or interstate movement of those cotton lines or their progeny. Importation of lepidopteran-resistant cotton and nursery stock or seeds capable of propagation is still, however, subject to the restrictions found in the Foreign Quarantine Notice regulations at 7 CFR Part 319. Variety registration and/or seed certification for individual cotton lines carrying the lepidopteran-resistant gene may involve future actions by the U.S. Plant Variety Protection Office and State Seed Certification officials.

The potential environmental impacts associated with this determination have been examined in accordance with regulations and guidelines implementing the National Environmental Policy Act of 1969 (42 U.S.C. 4321 *et seq.*; 40 CFR 1500-1509; 7 CFR Part 1b; 60 FR 6000-6005). An environmental assessment (EA) was prepared and a finding of no significant impact (FONSI) was reached by APHIS for the determination that lepidopteran-resistant cotton is no longer a regulated article under its regulations at 7 CFR Part 340. The EA and FONSI are available from APHIS upon written request or on the APHIS World Wide Web at http://www.aphis.usda.gov/BBEP/BP/.

The body of this document consists of two parts: (1) background information which provides the regulatory framework under which APHIS has regulated the field testing, interstate movement, and importation of lepidopteran-resistant cotton, as well as a summary of comments provided to APHIS on its proposed action; and (2) analysis of the key factors relevant to the APHIS decision that lepidopteran-resistant cotton does not present a plant pest risk.

II. Regulatory Background

USDA Regulatory Framework

APHIS regulations, which were promulgated according 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, regulate the introduction (importation, interstate movement, or release into the environment) of certain genetically engineered organisms and products. A genetically engineered organism is deemed a regulated article either if the donor organism, recipient organism, vector or vector agent used in engineering the organism belongs to one of the taxa listed in subsection 340.2 of the regulations and is also plant pest; if it is unclassified; or if APHIS has reason to believe that the genetically engineered organism presents a plant pest risk.

Prior to the introduction of a regulated article, a person is required under subsection 340.1 of the regulations to either (1) notify APHIS in accordance with subsection 340.3 or (2) obtain a permit in

accordance with subsection 340.4. Introduction under notification (subsection 340.3) requires that the introduction meets specified eligibility criteria and performance standards. The eligibility criteria impose limitations on the types of genetic modifications that qualify for notification, and the performance standards impose limitations on how the introduction may be conducted. Under subsection 340.4, a permit is granted for a field trial when APHIS has determined that conducting the field trial under the conditions specified by the applicant or stipulated by APHIS does not present a plant pest risk.

The FPPA gives USDA authority to regulate plant pests and other articles to prevent direct or indirect injury, disease, or damage to plants, plant products, and crops. The PQA provides an additional level of protection by enabling USDA to regulate the importation and movement of nursery stock and other plants which may harbor injurious pests or diseases and requires that they be grown under certain conditions after importation. For certain genetically engineered organisms, field testing may be required to verify that they exhibit the expected biological properties and although they were derived using components from plant pests, they do not possess plant pest characteristics.

An organism is not subject to the regulatory requirements of 7 CFR Part 340 when it is demonstrated not to present a plant pest risk. Section 340.6 of the regulations, entitled "Petition Process for Determination of Nonregulated Status", provides that a person may petition the Agency to evaluate submitted data and determine that a particular regulated article does not present a plant pest risk and should no longer be regulated. If the agency determines that the regulated article does not present a risk of introduction or dissemination of a plant pest, the petition will be granted, thereby allowing unregulated introduction of the article in question. A petition may be granted in whole or in part.

Lepidopteran-resistant cotton lines have been considered "regulated articles" for field testing under Part 340.0 of the regulations in part because the vector system used to transfer the bacterial *cryIA(c)* gene into the recipient cotton was derived from *A. tumefaciens*, which is on the list of organisms in the regulation and is widely recognized as a plant pathogen. In addition, certain noncoding regulatory sequences were derived from plant pathogens, i.e., from *A. tumefaciens* and from cauliflower mosaic virus, which are also on the list.

APHIS believes it is prudent to provide assurance prior to commercialization that organisms, such as the lepidopteran-resistant cotton, that are derived at least in part from plant pests, do not pose any potential plant pest risk. Such assurance may aid the entry of new plant varieties into commerce or into breeding and development programs. The decision by APHIS that the lepidopteran-resistant cotton is no longer a regulated article is based in part on evidence provided by Monsanto concerning the biological properties of the lepidopteran-

resistant cotton and its similarity to other varieties of cotton grown using standard agricultural practices for commercial sale or private use.

The fact that APHIS regulates genetically engineered organisms having plant pest components does not carry with it the presumption that the presence of part of a plant pest makes a whole plant a pest or that the plants or genes are pathogenic. The regulations instead have the premise that when plants are developed using biological vectors from pathogenic sources, they should be evaluated to assure that there is not a plant pest risk (McCammon and Medley, 1990). The goal of the APHIS regulations in the Code of Federal Regulations at 7 CFR Part 340 is to allow for the safe testing of genetically engineered organisms under an appropriate level of oversight which will allow any issues of potential or hypothetical risks to be addressed.

A determination that an organism does not present a plant pest risk means that there is reasonable certainty that the organism cannot directly or indirectly cause disease, injury, or damage, either when grown in the field, or when stored, sold, or processed. APHIS' approach to plant pest risk is considerably broader than a narrow definition that encompasses only plant pathogens. Other traits such as increased weediness and harmful effects on beneficial organisms, such as earthworms and bees, are clearly subsumed within what is meant by direct or indirect plant pest risk. In APHIS' regulations at 7 CFR Part 340, a "plant pest" is defined as: "Any living stage (including active and dormant forms) of insects, mites, nematodes, slugs, snails, protozoa, or other invertebrate animals, bacteria, fungi, other parasitic plants or reproductive parts thereof; viruses; or any organisms similar to or allied with any of the foregoing; or any infectious agents or substances, which can directly or indirectly injure or cause disease or damage in or to any plants or parts thereof, or any processed, manufactured, or other products of plants." A determination that an organism does not present a plant pest risk can be made under this definition, especially when there is evidence that the plant under consideration: (1) exhibits no plant pathogenic properties; (2) is no more likely to become a weed than its nonengineered parental varieties; (3) is not likely to increase the weediness potential for any other cultivated plant or native wild species with which the organism can interbreed; (4) does not cause damage to processed agricultural commodities; and (5) is not likely to harm other organisms, such as bees, that are beneficial to agriculture. Evidence has been presented by Monsanto regarding all of these points. In addition, because the Monsanto petition seeks a determination regarding new cotton varieties containing the *cryIA(c)* gene, it should be established that there is a reasonable certainty that any new cotton varieties bred from lepidopteran-resistant cotton lines will not exhibit plant pest properties substantially different from any observed for cotton in traditional breeding programs or as seen in the development of the lepidopteran-resistant cotton lines already field tested.

Oversight by Other Federal Agencies

The lepidopteran-resistant cotton lines are currently subject to regulations administered by the EPA and/or the FDA that require registration of pesticides prior to their distribution and sale and establish tolerances for pesticide residues in raw agricultural products. APHIS' decision on the regulatory status of the leptidopteran-resistant cotton, under APHIS' regulations at 7 CFR 340, in no way releases these cotton lines from EPA and FDA regulatory oversight.

Environmental Protection Agency (EPA).

The EPA regulates the use of pesticide chemicals, including insecticides, in the environment. Under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136 et seq.), EPA has the authority to regulate the testing, sale, distribution, use, storage, and disposal of pesticides. Before a pesticide may be sold, distributed, or used in the United States, it must be registered under FIFRA section 3. To be registered, FIFRA requires that a pesticide will not, when used in accordance with widespread and commonly recognized practice, cause "unreasonable adverse effects." In determining whether to approve the new use of the pesticide, EPA considers the possibility of adverse effects to human health and the environment from the new use. Accordingly, Monsanto has submitted to EPA an application for a registration for a transgenic plant pesticide containing the new active ingredient *Btk* δ-endotoxin protein as produced by the *cryIA(c)* gene and its controlling sequences. On February 15, 1994, EPA announced receipt of this application (EPA File Symbol 524-UTI) in the Federal Register (OPP-30373; FRL-4913-5, 59 FR 49663).

Under the Federal Food, Drug and Cosmetic Act (FFDCA) (21 U.S.C. 201 et seq.), EPA also has responsibility for establishing tolerances for pesticide residues on food or feed. Pesticides added to raw agricultural commodities generally are considered to be unsafe unless a tolerance or exemption from tolerance has been established. Foods containing unsafe pesticides are deemed to be adulterated. Residue tolerances for pesticides are established by EPA under the Federal Food, Drug, and Cosmetic Act; the FDA enforces the tolerances set by the EPA. Monsanto has also submitted to the EPA a pesticide petition (PP 4F 4331) proposing to amend 40 CFR part 180 to establish a tolerance exemption for residues of the plant pesticide active ingredient *Bacillus thuringiensis* subsp. *kurstaki* δ-endotoxin protein as produced by the *cryIA(c)* gene and its controlling sequences. Consistent with the "Coordinated Framework for Regulation of Biotechnology" (51 FR 23302-23350, June 26, 1986), APHIS and the EPA are coordinating their review of these genetically engineered cotton lines to avoid duplication and assure that all relevant issues are addressed.

Food and Drug Administration (FDA).

The FDA policy statement concerning regulation of plants derived from new plant varieties was published in the Federal Register on May 29, 1992, and appears at 57 FR 22984-23005. Food safety in the United States, for products other than meat and poultry, is assured by regulation under the FFDCA. FDA's policy statement concerning the regulation of foods derived from new plant varieties, including genetically engineered plants, was published in the Federal Register on May 29, 1992, and appears at 57 FR 22984-23005. Regulatory oversight for the safety of any food or feed products derived from lepidopteran-resistant cotton is under the jurisdiction of the FDA.

III. Public Comments

Summary of Comments

APHIS received 69 comments on the subject petition from cotton farmers (19), individuals (14), universities (12), agricultural experiment stations (8), cooperative extension services (7), U.S. Department of Agriculture, Agricultural Research Service (3), a bank, a chemical company, a cotton researcher, a cotton cooperative association, a gas and oil supplier, and a worker's compensation trust. Sixty-eight of the commenters either expressed support for the petition and provided information in support of nonregulated status for Monsanto's insect-resistant cotton variety or urged expedited approval to allow commercial planting of the lepidopteran-resistant cotton lines. Among the general points made by the commenters urging approval of the petition were the need for the product because of the critical problem of budworm and bollworm control in cotton production, the anticipated reduction in pesticide use and resulting cost reduction, public health and environmental benefits, and an increased ability to utilize a variety of integrated pest management strategies with the insect-resistant cotton variety. The specific points stressed by commenters providing information in support of the petition included observations of the subject cotton lines' efficacy against targeted pests and the resulting potential for an improvement in the options available for control of *Heliothis/Helicoverpa* moths in cotton. Several commenters addressed the need for a sound resistance management strategy and expressed support for the Monsanto Company's attempts to develop and implement such a strategy.

One commenter, who found the subject petition "generally competent," neither urged its approval or denial, but cited three issues "deserving more careful consideration": (1) the effects of long-term release of *Btk* protein on the soil ecosystem; (2) the durability of *Btk* effectiveness in the face of pest evolution [pesticide resistance] when the only strategy in place is reliance on "high expression of toxin" to kill insects heterozygous for resistance; and (3) the potential severe economic costs resulting from widespread adoption of *Btk* crops in the absence of effective resistance management strategies.

APHIS Response to Comments.

APHIS concurs that the three issues raised by the commenter merit consideration. We note, however, that Monsanto has attempted to address each of these issues. Our analysis is as follows.

(1) Monsanto has conducted some field tests with lepidopteran-resistant cotton in the same test plots for up to 7 consecutive years at multiple sites in Lousiana, Texas, and Mississippi. Each year, the field tests were terminated by disking/tilling the plants into the soil. The researchers monitoring the field reported no detrimental effects on soil fertility or changes in beneficial insect populations (Dr. John H. Benedict, Texas A & M University; William Caldwell, Louisiana Agricultural Experiment Station; and Dr. Johnie Jenkins, USDA Agricultural Research Service, Starkville, MS; personal communications). Studies performed by Monsanto also reported decay rates of the insect control protein in the lepidopteran-resistant cotton to be comparable to microbial *Btk* formulations. APHIS believes that growing the lepidopteran-resistant cotton on a commercial scale will not have significant detrimental effects on the soil ecosystem.

(2) and (3) APHIS concludes that development of resistance to insecticides is a potential risk associated with their use; but in this respect, cultivation of lepidopteran-resistant cotton should have no greater impact on the control of lepidopteran pests in cotton and other crops than the widely practiced method of applying insecticides to control insects on cotton. The implementation of an active resistance management plan that is scientifically sound and acceptable to growers should delay the onset of resistance to lepidopteran-resistant cotton and provide alternative strategies and methods for managing or containing resistant lepidopteran populations, if and when they arise. Product labels and informational brochures should help define the appropriate use of this cotton and reduce any potential risks associated with their use. Monsanto has conducted extensive research on resistance management and met with APHIS to discuss their strategies. To paraphrase the thoughts of several commenters, Monsanto proposes a resistance management program that is as scientifically sound as can be developed at this time.

IV. Analysis of the Properties of Lepidopteran-Resistant Cotton

Brief discussions of the biology of cotton and of cotton cultivation practices follow in the next paragraph to help inform the subsequent analysis. This information is expanded in subsequent sections when it is relevant in addressing particular issues with respect to lepidopteran-resistant cotton.

Biology and Cultivation of Cotton

Cotton is grown primarily for the seed hairs that are made into textiles. Cotton is predominant as a textile fiber because the mature dry hairs twist in such a way that fine, strong threads can be spun

from them. Other products, such as cottonseed oil, cake, and cotton linters are byproducts of fiber production. Cotton, a perennial plant cultivated as an annual, is grown in the United States mostly in areas from Virginia southward and westward to California, in a region often referred to as the Cotton Belt (McGregor, 1976).

Cotton belongs to the genus *Gossypium*, which includes 39 species, four of which are generally cultivated (Fryxell, 1984). The most commonly cultivated species, *G. hirsutum* L., is the subject of this petition. Other cultivated species are *G. arboreum* L., *G. barbadense* L., and *G. herbaceum* L.

Four species of *Gossypium* occur in the United States (Fryxell, 1979; Kartesz and Kartesz, 1980). *G. hirsutum* is the primary cultivated cotton. *G. barbadense* is also cultivated. The other two species, *G. thurberi* Todaro and *G. tomentosum* Nuttall ex Seemann, are wild plants of Arizona and Hawaii, respectively. *G. tomentosum* is known from a few strand locations very close to the ocean.

At least seven genomes (chromosome sets with distinctive gene groupings), designated A, B, C, D, E, F, and G, are found in the genus (Endrizzi, 1984). Diploid species (2n=26) are found on all continents, and a few are of some agricultural importance. The A genome is restricted in diploids to two species (*G. arboreum*, and *G. herbaceum*) of the Old World. The D genome is restricted in diploids to some species of the New World, such as *G. thurberi*.

By far, the most important agricultural cottons are *G. hirsutum* and *G. barbadense*. These are both allotetraploids (plants with four sets of chromosomes derived by doubling of chromosomes from a hybrid plant) of New World origin, and presumably of ancient cross between Old World A genomes and New World D genomes. The simplest forms of these plants have 52 chromosomes, and are frequently designated as AADD. Four additional New World allotetraploids occur in the genus, including *G. tomentosum*, the native of Hawaii. *G. tomentosum*, *G. hirsutum*, and *G. barbadense* have compatible genome types, and can be crossed to produce viable offspring, although crosses with *G. tomentosum* are only known with certainty from artificial crosses in breeding programs. *G. thurberi* does not successfully cross with the allotetraploids.

G. hirsutum is generally self-pollinating, but in the presence of suitable insect pollinators can exhibit cross-pollination. Bumblebees (*Bombus* spp.), *Melissodes* bees, and honey bees (*Apis mellifera*) are the primary pollinators (McGregor, 1976). The concentration of suitable pollinators varies by location and season and is considerably suppressed by insecticide use. Even if suitable bee pollinators are present, the distribution of pollen decreases considerably with increasing distance. The isolation distances for Foundation, Registered, and Certified seed in 7 CFR Part 201 are 1320 feet, 1320 feet, and 660 feet, respectively.

The growing period for cotton, from planting until removal of the last harvestable cotton boll, ranges from 140 to 200 days and depends on the planting site in the Cotton Belt (El-Zik et al., 1989). Cotton as a crop is highly susceptible to attack by insects and plant pathogens. Programs requiring particular management practices to combat particular cotton pests are in place in some states. For example, State programs for pink bollworm (*Pectinophora gosspiella)* management in the Southwest require that the mature crop be defoliated or desiccated and that stalks be shredded and plowed into the soil to prevent overwintering of the insect.

Insect management is a major concern in the cultivation of cotton with the major pests belonging in the Order lepidoptera (moths, butterflies and skippers). The main pests are the cotton bollworm (*Helicoverpa zea*), the tobacco budworm (*Heliothis verescens*) and the pink bollworm (*P. gosspiella*). These insects infest approximately 10.4 million acres of cotton in the U.S., and approximately $180 million is spent annually for their control.

V. Conclusions regarding the properties of lepidopteran-resistant cotton.

To reach the determination that lepidopteran-resistant cotton does not present a plant pest risk, APHIS analyzed public comments, basic information on the biology of cotton, data presented by Monsanto, and other relevent scientific data pertaining to the petition. Based on the data described, APHIS has arrived at a series of conclusions regarding the properties of lepidopteran-resistant cotton.

A. The introduced genes, their regulatory sequences and their products do not present a plant pest risk in the lepidopteran-resistant cotton lines.

Three lepidopteran-resistant cotton lines (531, 757, and 1076) were produced using an *Agrobacterium*-mediated transformation protocol. The Coker 312 line was transformed with a plasmid containing the *cryIA(c)* gene, which confers resistance to some lepidoptera, and the *nptII* gene, which confers resistance to the antibiotic kanamycin. The gene for lepidopteran resistance, *cryIA(c)* (Höfte and Whitely, 1989) was isolated from *B. thuringiensis* subsp. *kurstaki* (*Btk*) strain HD-73, which is not a regulated article. Subspecies of the gram-positive soil bacterium *B. thuringiensis* are characterized by their ability to produce crystalline inclusion proteins (δ-endotoxins) which have highly specific insecticidal properties. This *cryIA(c)* gene was constructed based on the first 1398 nucleotides of the *cryIA(b)* gene (Fischhoff et al., 1987) with nucleotides 1399 to 3524 of the *cryIA(c)* gene (Adang et al., 1985). The nucleotide sequence was modified for plant codon preferences. With the exception of 7 amino acids, the *cryIA(c)* gene product is identical to the analogous region of the CryIA(c) protein found in nature (Adang et al., 1985). The CryIA(c) protein produced in the transgenic plant yields an insecticidally active trypsin-resistant core product identical to the native CryIA(c)

protein. Upon ingestion of this protein by susceptible insects, feeding is inhibited by disruption of the midgut epithelium, which eventually results in death. For a review of Cry insecticidal proteins, see Höfte and Whitley, 1989.

The gene encoding the protein neomycin phosphotransferase type II (also called NPTII or aminoglycoside 3'-phosphotransferase II) was isolated from a transposon contained in a strain of *E. coli* K12 (Beck et al., 1982; Jorgensen et al., 1979). *E. coli*, a common enteric bacterium found in the gut of animals, is not a regulated article. The gene has no involvement in plant disease or damage. This gene was introduced as a marker, i.e., as a tag enabling selection of cotton cells that had taken up the *cryIA(c)* gene. Following transformation, plant cells expressing the enzyme NptII can grow in the presence of the antibiotic kanamycin because NptII deactivates, by phosphorylation, aminoglycoside antibiotics such as kanamycin. Its use does not result in the presence of the antibiotic kanamycin in lepidopteran-resistant cotton, and its presence does not imply that kanamycin will be used in the cultivation of cotton.

These cotton lines also contain the *aad* bacterial marker gene which encodes 3'(9)-O-aminoglycoside adenylyltransferase. This gene was isolated from the Tn7 bacterial transposon and provides resistance to the antibiotics spectinomycin and streptomycin. This marker gene is only used to select for bacterial strains that have been transformed with the desired plasmid. The gene product is not expressed in the plant.

The introduced DNA that contains the *cryIA(c)* and *nptII* genes also has accompanying DNA regulatory sequences that regulate the expression of these genes in plants. The DNA regulatory sequences were derived from a nonpathogenic organism, *Pisum sativum* (pea), and two organisms which are plant pathogens: the bacterium *A. tumefaciens* and cauliflower mosaic virus (CaMV). Another regulatory region, which has been claimed as Confidential Business Information, was also used. Specifically, the DNA regulatory sequences associated with the *cryIA(c)* gene comprise the promoter and terminator. The promoter is derived from the 35S gene of CaMV with a duplicated enhancer region (Kay et al., 1987; Odell et al., 1985). The terminator was derived from either the 3' nontranslated region of the pea ribulose-1,5-bisphosphate carboxylase, small subunit (*rbcS*) E9 gene (Coruzzi et al., 1984) or the 3' non-translated region of the soybean alpha subunit of the beta-conglycinin gene (Schuler *et al.*, 1982) which functions to terminate transcription and direct polyadenylation of the *cryIA(c)* mRNA. The DNA regulatory sequences associated with the *nptII* gene comprise the CaMV 35S promoter (Gardner et al., 1981; Sanders et al., 1987) and the 3' nontranslated region of the nopaline synthase gene from *A. tumefaciens*, which functions to terminate transcription and direct polyadenylation of the *nptII* gene (Depicker et al., 1982; Bevan et al., 1983). Although these regulatory sequences were derived from plant pathogens, the regulatory sequences cannot cause plant disease by themselves or with the genes that they regulate. The genes

and their regulatory sequences were subcloned between the left and right T-DNA borders of an *A. tumefaciens* binary-plasmid transformation vector.

Lepidopteran-resistant cotton lines were derived by transforming the Coker 312 cotton line with the previously described plasmid vector via a well-characterized technique that uses DNA sequences from *A. tumefaciens* to introduce those genes subcloned between the T-DNA borders into the chromosome of the recipient plant (see reviews by Klee and Rogers, 1989; and Zambryski, 1988). Although some DNA sequences used in the transformation process were derived from the plant pathogen *A. tumefaciens* (the causal agent of crown gall disease), the genes that cause crown gall disease were removed; therefore, the cotton plant does not develop crown gall disease. Once inserted into the chromosome of the transformed plant, the introduced genes are maintained and sexually-transmitted in the same manner as any other genes.

Analyses of the different lepidopteran-resistant cotton lines indicated that one complete copy of the *cryIA(c)* and the *nptII* gene along with a partial copy of the *cryIA(c)* gene was inserted into and stably maintained in the chromosomal DNA. The accumulation of the CryIA(c) protein in the cotton plant was determined by enzyme-linked immunosorbent assays. The amount of the CryIA(c) insect control protein in the leaf tissue had mean (S.D.) values 1.6 (0.15), 12.7 (2.5), and 12.2 (2.5) μg protein/ g fresh weight for lines 531, 757, and 1076, respectively. The amount of the CryIA(c) measured in the seed was 0.86 (0.18), 9.8 (1.3), and 12.7 (0.6) μg protein/ g fresh weight for lines 531, 757, and 1076, respectively. Generally, the CryIA(c) protein level varied less than five-fold in young leaf tissue assayed over the growing season. Compared to CryIA(c) protein, the mean accumulation of NptII protein across all lines and sites was at comparable or lower levels. Therefore, these genes are expressed constitutively in the transgenic cotton plant.

During extensive field testing, the lepidopteran-resistant cotton lines exhibited agronomic properties typical of the recipient Coker 312 nontransgenic control, with the exception of the desired lepidopteran-resistant phenotype conferred by the CryIA(c) protein. APHIS believes that the components, quality and processing characteristics of lepidopteran-resistant cotton reveal no differences with nontransgenic cotton which would indicate any direct or direct plant pest property of the new cotton lines. The lepidopteran-resistant cotton exhibits no plant pest characteristics.

B. Lepidopteran-resistant cotton has no significant potential to become a successful weed.

Almost all definitions of weediness stress as core attributes the undesirable nature of weeds from the point of view of humans. Individual definitions differ in approach and emphasis (Baker, 1965; de Wet and Harlan, 1975; Muenscher, 1980). Baker (1965) listed 12

common weed attributes, almost all pertaining to sexual and asexual reproduction, which can be used as an imperfect guide to the likelihood that a plant will behave as a weed. Keeler (1989) and Tiedje et al. (1989) have analyzed and adapted Baker's list to develop admittedly imperfect guides to the weediness potential of transgenic plants. These authors emphasize the importance of looking at the parent plant and the nature of the specific genetic changes.

The parent plant in this petition, *G. hirsutum*, does not show any appreciable weedy characteristics. The genus also seems to be devoid of any such characteristics. Although some New World allotetraploid cottons show tendencies to "weediness" (Fryxell, 1979; Haselwood et al., 1983), the genus shows no particular weedy aggressive tendencies. The standard texts and lists of weeds give no indication that cotton is clearly regarded as a weed anywhere (Holm et al., 1979; Muenscher, 1980; Reed, 1970; Weed Science Society of America, 1989). Any reports that cottons behave as weeds are rare, anecdotal, and vague as to the nature of the problem.

Examination of the new genetic sequences including the gene encoding lepidopteran resistance that may be present in the transgenic plant shows no likelihood to increase weediness potential. None of these confers any traits in any way associated with weediness.

Monsanto's data from greenhouse and field studies show no significant differences in germination rates or stand counts among transgenic and control cotton lines. No obvious increase in volunteer plants was noted following field trials at multiple sites.

C. Lepidopteran-resistant cotton will not increase the weediness potential of any other plant with which it can interbreed.

As with *G. hirsutum*, discussed in section B above, neither *G. barbadense*, *G. thurberi*, nor *G. tomentosum* show any definite weedy tendencies.

Movement of genetic material by pollen is possible only to those plants of a compatible chromosomal type, in this instance only to those allotetraploid cottons with AADD genomes. In the United States, this would include *G. hirsutum*, *G. barbadense*, and *G. tomentosum*. Lepidopteran-resistant cotton is chromosomally compatible with wild *G. hirsutum*. According to Dr. Paul Fryxell of Texas A&M University (personal communication), a leading authority on the systematics and distribution of Gossypieae, wild cottons are found only in southern Florida (virtually exclusively in the Florida Keys). In contrast, cultivated cottons are found in northernmost portions of the state. Other wild *G. hirsutum* found around the Gulf of Mexico occurs along the Mexican coast and largely along the Yucatan peninsula. Populations do not extend as far north as the Texas border. Even if the nonagricultural land containing these wild cotton populations were near sites of commercial cotton production, this determination would not be altered because: (1) any potential effects of the trait would

not alter the weediness of the wild cotton; and (2) the wild cotton populations in Florida are not being actively protected and have been subject to Federal eradication campaigns because they can serve as potential hosts for the boll weevil, *Anthonomus grandis* Boh.

Gossypium thurberi, the native diploid from Arizona with a DD genome, is not compatible with *G. hirsutum* pollen, so that lepidopteran-resistant cotton can have no effect on this species. Movement to *G. hirsutum* and *G. barbadense* is possible if suitable insect pollinators are present, and if there is a short distance from transgenic plants to recipient plants. Any physical barrier, intermediate pollinator-attractive plants, or other temporal or biological impediments would reduce the potential for pollen movement.

Movement of genetic material to *G. tomentosum* is more speculative. The wild species is chromosomally compatible with *G. hirsutum*, but there is uncertainty about the possibility for pollination. The flowers of *G. tomentosum* seem to be pollinated by moths, not bees, and they are reportedly receptive at night, not in the day. Both these factors greatly lessen the probability of cross-pollination. Fryxell (1979) reported that *G. tomentosum* may be losing its genetic identity from introgressive hybridization of cultivated cottons by unknown means. Additionally, Stephens (1964) reported probable hybrid populations of *G. barbadense* X *G. tomentosum*, in a study of morphological attributes. However, DeJoode and Wendel (1992) indicated that despite the morphological suggestion of such hybrid populations, biochemical (allozyme) studies show no evidence of any such introgression, even with the presence of clear species-specific allozyme alleles. Major factors influencing the survival of *G. tomentosum* are construction and urbanization, i.e., habitat destruction (Fryxell, 1979). APHIS believes that it is these factors, rather than gene introgression from cultivated cottons, that are of real significance to this species. Cotton lines bred by traditional means, which should be no more or less likely to interbreed with *G. tomentosum* than lepidopteran-resistant cotton are not considered to pose a threat to the wild cotton and are not subject to particular State or Federal regulation on this basis. Neither the weediness nor the survival of *G. tomentosum*, therefore, will be affected by the cultivation of lepidopteran-resistant cotton because the transgenic variety poses no increased weediness itself and the two species are unlikely to successfully cross in nature.

In contrast to the situation with *G. tomentosum*, gene movement from *G. hirsutum* to *G. barbadense* is widespread in advanced cultivated stocks. However, it is conspicuously low or absent in material derived from natural crosses such as that from Central America or the Caribbean where *G. hirsutum* and *G. barbadense* grow together. The absence of natural introgression may be caused by any one of several isolating mechanisms of pollination, fertilization, ecology, gene incompatibility, or chromosome incompatibility (Percy and Wendel, 1990). Movement of gene material from lepidopteran-resistant cotton to cultivated or occasional noncultivated *G. barbadense* would

therefore not likely occur at a high level. Any movement of genetic material from lepidopteran-resistant cottons into *G. barbadense* is likely to be the result of intentional breeding practice rather than accidental crossing.

D. Lepidopteran-resistant cotton will not cause damage to processed agricultural commodities.

Cottonseed is processed into four major products: oil, meal, hulls and linters (Cherry and Leffler, 1984). Information provided by Monsanto regarding the components and processing characteristics of lepidopteran-resistant cotton revealed no differences in any component that could have an indirect plant pest effect on any processed plant commodity. Monsanto evaluated the effects of the genetic modifications on lepidopteran-resistant cotton by measuring fiber characteristics, seed processing characteristics, and the biochemical composition of oil and meal. For the evaluation of seed processing characteristics, Monsanto presented data on the processing of delinted seeds from three lines of lepidopteran-resistant cotton and the Coker 312 control. Although these data show some variability among the lines tested, the results were comparable to those from the control plants.

E. Lepidopteran-resistant cotton will not be harmful to beneficial organisms, including bees.

Consistent with its statutory authority and requirements under NEPA, APHIS evaluated the potential for lepidopteran-resistant cotton plants, plant products, and the *Btk* insect control protein to have damaging or toxic effects directly or indirectly on nontarget organisms, particularly those that are recognized as beneficial to agriculture. APHIS also considered potential impacts on other nontarget pests, since such impacts could affect the potential for changes in agricultural practices. There is no reason to believe that the NptII protein conferring kanamycin resistance in the transgenic cotton lines as a selectable marker for transformation would have deleterious effects or significant impacts on nontarget organisms, including beneficial organisms. There have been no reports of toxic effects on such organisms in the many field trials conducted with many different plants expressing this selectable marker.

1) Potential impact on beneficial organisms

Microbial formulations of crystalline insecticidal proteins from *Bacillus thuringiensis* have been used for control of agricultural insect pests for over 25 years (Feitelson, 1992). The formulations which include bacterial strains producing the CryIA(c) protein are very selective for lepidopteran insect (MacIntosh et al., 1990; Klausner, 1984; Hoffmann et al., 1988, Dulmage, 1981; Whitely and Schnepf, 1986). These microbial *Bt* formulations have been shown to have no deleterious effect on beneficial insects including predators and parasitoids of lepidopteran pests or honeybees. For a review of

microbial *Bt* formulations, see Vinson (1990) and Melin and Cozzi (1990).

Monsanto provided data to demonstrate that the lepidopteran-resistant cotton lines express a product that, as expected, is similar in molecular weight and immunological reactivity to the crystalline endotoxin found in the microbial Bt formulations. Thus, the specificity of the lepidopteran-resistant CryIA(c) protein would not be expected to differ from the microbial *Btk* products. For nontarget organisms to be affected, these organisms would have to feed on the cotton, thereby making them cotton pests, or they would have to feed on other cotton pests. To confirm the specificity of the Btk protein produced in lepidopteran-resistant cotton lines, Monsanto assessed the potential toxicity of the lepidopteran-resistant *Btk* protein to ten insect species from five different orders of insects, including Lepidoptera. Only four species of Lepidoptera, which are cotton pests, were sensitive to either the full-length or the trypsin-resistant core lepidopteran-resistant Btk protein. No toxic effects were observed on beneficial insects including parasitic Hymenoptera (*Nasonia vitripennis*), adult honey bee (*Apis mellifera* L.), ladybird beetles (*Hippodamia convergens*) or green lacewing larvae (*Chrysopa carnea*). Furthermore, in the 125 field test sites conducted by experienced cotton breeders and entomologists, non-target insect populations were monitored for susceptibility to lepidopteran-resistant cotton lines; no differences in insect toxicity were observed between the transgenic and non-transgenic cotton lines.

2) Potential impacts on other nontarget organisms

Other invertebrates, such as earthworms, and all vertebrate organisms including nontarget birds, mammals and humans are not expected to be affected by the CryIA(c) protein because they do not contain the receptor protein found in the midgut of target insects. Moreover, exposure of fish and wildlife to the transgenic cotton is likely to be minimal. Cotton is a unique field crop in that mammals and other species avoid feeding on the plant due to both the gossypol content and the morphology of the plant. Avian species are not expected to feed on the lint-covered seed found in fields after harvest. Seed and plant debris is not expected to enter aquatic habitats where fish would be exposed. Nevertheless, Monsanto conducted to assess the wholesomeness of lepidopteran-resistant cottonseed meal when fed to the bobwhite quail. No mortality occurred in birds fed up to 100,000 ppm raw cotton seed meal in their diet. The level of feeding approximates 400 seeds/kg body weight per bird per day.

APHIS concludes that lepidopteran-resistant cotton exhibits no significant potential to adversely impact organisms which are beneficial to plants or agriculture. Furthermore, the cotton will not adversely impact the ability to control nontarget insect pests of agriculture.

F. Impacts on the current agricultural practices

Integrated Pest Management. APHIS considered potential impacts associated with the cultivation of lepidopteran-resistant cotton on the current agricultural practices used to control lepidopteran cotton pests. An article included in the Petition discussed the impact of lepidopteran-resistant cotton on cotton pest management: "Impact of Transgenic Cotton Expressing Endotoxin Proteins from *Bacillus thuringiensis* on Cotton Insect Management in the USA" by R.G. Luttrell (Mississippi State University), J.H. Benedict (Texas A&M University), G.A. Herzog (University of Georgia), and T.F. Watson (University of Tucson). The authors supported the use of Bt cotton, and they were encouraged by opportunities that the transgenic Bt cotton would provide in integrated pest management. APHIS agrees with the authors that lepidopteran-resistant cotton provides unique opportunities in integrated pest management, as discussed below, that are not possible when using broad-spectrum, nerve toxin insecticides.

The CryIA(c) protein produced in the lepidopteran-resistant cotton is an environmentally safe and effective means to control the tobacco budworm-bollworm complex and pink bollworm. Topical applications of the CryIA(c) protein in microbial formulations for pest control on several crops has been approved by the EPA. Expression of this insect control protein in the plant provides continuous control of the insects, provides control independently of weather conditions, and provides control in areas of the plant (such as below the canopy) where aerial application is less effective.

The insect control protein is very selective for certain lepidopteran species and has no effect on agriculturally beneficial insects. Traditional insecticides are less selective than Bt insecticides and reduce natural beneficial populations. Survival of naturally occurring beneficial insects may allow for control of some insect pests through natural predation. However, because some nonlepidopteran pests have been controlled indirectly through insecticide applications for lepidopteran pests, these insect populations should be monitored more closely and controlled, if necessary.

Resistance Management. Monsanto also submitted a summary of their strategy for maximizing the utility of these plants and delaying the development of insects resistant to the *Btk* insect control protein. Their strategy was also submitted to the EPA in support of the registration of lepidopteran-resistant cotton as a plant pesticide. APHIS reviewers met with EPA's Pesticide Resistance Management Workgroup to discuss their evaluation of this strategy and offer comments.

The Monsanto resistance management plan includes:

1. high dose expression,
2. use of refugia,
3. monitoring insect population for resistance to *Btk*,
4. using agronomic practices that minimizes insect exposure to *Btk* plants,
5. integrated pest management, and
6. pyramiding or stacking of multiple resistant genes within the same plant.

The effectiveness of a resistance management plan cannot truly be evaluated until the plants are grown on a commercial scale. However, Monsanto has described a reasonable plan that is consistent with the information currently available. The development of effective resistance management strategies is an ongoing process, and when appropriate, APHIS will offer comments and recommendations for technical improvements to the EPA and Monsanto to assist in this process. The EPA is evaluating Monsanto's resistance management strategy and has stated that they are committed to working with Monsanto to develop a sound plan that is consistent with current resistance management strategies. EPA must grant pesticide registration of lepidopteran-resistant cotton before the plant can be grown commercially.

VI. Conclusion

APHIS has determined that cotton plants fitting the definition of Bollgard™ lepidopteran-resistant cotton, that have previously been field tested under permit or notification, will no longer be considered regulated articles under APHIS regulations at 7 CFR Part 340. Permits under those regulations will no longer be required from APHIS for field testing, importation, or interstate movement of those cotton lines or their progeny. (Importation of lepidopteran-resistant cotton [and nursery stock or seeds capable of propagation] is still, however, subject to the restrictions found in the Foreign Quarantine Notice regulations at 7 CFR Part 319.) This determination has been made based on an analysis which revealed that those cotton lines: (1) exhibit no plant pathogenic properties; (2) are no more likely to become a weed than their nonengineered parental varieties; (3) are not likely to increase the weediness potential for any other cultivated plant or native wild species with which the organisms can interbreed; (4) will not cause damage to processed agricultural commodities; and (5) are not likely to harm other organisms, such as bees, that are beneficial to agriculture. APHIS has also concluded that there is a reasonable certainty that new lepidopteran-resistant cotton varieties bred from these lines will not exhibit new plant pest properties, i.e., properties substantially different from any observed for the lepidopteran-resistant cotton lines already field tested, or those observed for cotton in traditional breeding programs.

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