DEPARTMENT OF AGRICULTURE

Animal and Plant Health Inspection Service

[Docket No. 97–006–2]

Calgene, Inc.; 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 Calgene, Inc., cotton lines designated as BXN® with Bt cotton lines derived from transformation events 31807 and 31808 which have been genetically engineered for tolerance to the herbicide bromoxynil and resistance to lepidopteran insect pests, 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 Calgene, Inc., in its petition for a determination of nonregulated status and an analysis of other scientific data. This notice also announces the availability of our written determination document and its associated environmental assessment and finding of no significant impact.

EFFECTIVE DATE: April 30, 1997.

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

FOR FURTHER INFORMATION CONTACT: Dr. James White, BSS, PPQ, APHIS, 4700 River Road Unit 147, Riverdale, MD 20737–1236; (301) 734–8761. To obtain a copy of the determination or the environmental assessment and finding of no significant impact, contact Ms. Kay Peterson at (301) 734–4885; e-mail: mkpeterson@aphis.usda.gov.

SUPPLEMENTARY INFORMATION:

Background

On January 13, 1997, the Animal and Plant Health Inspection Service (APHIS) received a petition (APHIS Petition No. 97–013–01p) from Calgene, Inc., (Calgene) of Davis, CA, seeking a determination that cotton lines designated as BXN® with Bt cotton lines derived from transformation events 31807 and 31808 (events 31807 and 31808), which have been genetically engineered for bromoxynil herbicide tolerance and lepidopteran insect pest resistance, do not present a plant pest risk and, therefore, are not regulated articles under APHIS' regulations in 7 CFR part 340.

On February 21, 1997, APHIS published a notice in the **Federal Register** (62 FR 7996–7997, Docket No. 97–006–1) announcing that the Calgene 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 that notice, APHIS solicited written comments from the public as to whether these cotton lines posed a plant pest risk. The comments were to have been received by APHIS on or before April 22, 1997. During the designated 60-day comment period, APHIS received no comments on the subject petition.

Analysis

Events 31807 and 31808 have been genetically engineered to express a nitrilase enzyme isolated from *Klebsiella pneumoniae* subsp. *ozaenae*, which degrades the herbicide bromoxynil, and a CryIA(c) insect control protein originally derived from the common soil bacterium *Bacillus thuringiensis* subsp. *kurstaki* HD–73 (Bt). The subject cotton lines also express the *nptII* gene, which codes for the enzyme neomycin phosphotransferase and has been used as a selectable marker in the development of the transgenic cotton plants. Expression of the added genes is controlled in part by noncoding DNA sequences derived from the plant pathogens *Agrobacterium tumefaciens* and cauliflower mosaic virus. The *Agrobacterium* transformation method was used to transfer the added genes into the Coker 130 parental cotton plants.

The subject cotton lines have been considered regulated articles under APHIS' regulations in 7 CFR part 340 because they contain gene sequences derived from plant pathogens. However, evaluation of field data reports from field tests of the cotton lines conducted under APHIS notifications since 1994 indicates that there were no deleterious effects on plants, nontarget organisms, or the environment as a result of the environmental release of events 31807 and 31808.

Determination

Based on its analysis of the data submitted by Calgene and a review of other scientific data and field tests of the subject cotton plants, APHIS has determined that events 31807 and 31808: (1) Exhibit no plant pathogenic properties; (2) are no more likely to become weeds than cotton lines developed by traditional breeding techniques; (3) are unlikely to increase the weediness potential for any other cultivated or wild species with which they can interbreed; (4) will not cause damage to raw or processed agricultural commodities; (5) will not harm threatened or endangered species or other organisms, such as bees, that are beneficial to agriculture; and (6) should not reduce the ability to control insects in cotton or other crops when cultivated. Therefore, APHIS has concluded that the subject cotton lines and any progeny derived from hybrid crosses with other nontransformed cotton varieties will be as safe to grow as cotton in traditional breeding programs that are not subject to regulation under 7 CFR part 340.

The effect of this determination is that Calgene's cotton events 31807 and 31808 are no longer considered regulated articles under APHIS' regulations in 7 CFR part 340. Therefore, the 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, importation of cotton events 31807 and 31808 or seeds capable of propagation are 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, as amended (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 Calgene's cotton events 31807 and 31808 and lines developed from them are no longer regulated articles under its regulations in 7 CFR part 340. Copies of the EA and the FONSI are available upon request from the individual listed under **FOR FURTHER INFORMATION CONTACT**.

Done in Washington, DC, this 14th day of May 1997.

Donald W. Luchsinger,

Acting Administrator, Animal and Plant Health Inspection Service.

[FR Doc. 97-13116 Filed 5-19-97; 8:45 am]

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USDA/APHIS Petition 97-013-01p for Determination of Nonregulated Status for Events 31807 and 31808 Cotton

Environmental Assessment and
Finding of No Significant Impact

April 1997

The Animal and Plant Health Inspection Service (APHIS) of the U. S. Department of Agriculture has prepared an environmental assessment before issuing a determination of nonregulated status for a genetically engineered cotton called events 31807 and 31808 Cotton. These cotton plants have been engineered to be lepidopteran insect resistant and bromoxynil tolerant. APHIS received a petition from the Calgene, Inc., regarding the status of these events as regulated articles under APHIS regulations at 7 CFR Part 340. APHIS has conducted an extensive review of the petition, supporting documentation, and other relevant scientific information. Based upon the analysis documented in this environmental assessment, APHIS has reached a finding of no significant impact on the environment for its determination that events 31807 and 31808, therefore, these cotton plants shall no longer be regulated articles.

John H. Payne, Ph.D.
Director
Biotechnology and Scientific Services
Plant Protection and Quarantine
Animal and Plant Health Inspection Service
U.S. Department of Agriculture
Date: APR 30 1997

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I. SUMMARY

USDA/APHIS has prepared an Environmental Assessment in response to a petition (APHIS Number 97-013-01p) from Calgene, Inc., regarding designated events 31807 and 31808 cotton (*Gossypium hirsutum*). Calgene seeks a determination from APHIS that events 31807 and 31808 cotton do not present a plant pest risk and are therefore no longer regulated articles. The significant modifications to the 31807 and 31808 cotton plants relative to traditional cotton varieties are that events 31807 and 31808 cotton have been modified to express one genetic marker gene, a gene that provides tolerance to herbicide bromoxynil, and a gene that provides resistance to lepidopteran insects.

Events 31807 and 31808 cotton have previously been field tested under nine APHIS notifications. All field trials were performed under conditions of reproductive confinement.

This EA specifically addresses the potential for impacts to the human environment through the use in agriculture of events 31807 and 31808 cotton. It does not address the separate issue of the potential use of the herbicide bromoxynil (Buctril™) or the use of plant pesticide CryIA(c) in conjunction with events 31807 and 31808 cotton. The United States Environmental Protection Agency (EPA) has authority over the use in the environment of all pesticidal substances, including herbicides and insecticides, under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA).

The APHIS review and analysis of Calgene's petition in this EA result in a Finding of No Significant Impact (FONSI) to the human environment relative to the determination that events 31807 and 31808 cotton plants that have been previously field tested under the regulations at 7 CFR Part 340 will no longer be regulated articles.

II. BACKGROUND

A. Development of Events. Calgene has submitted a "Petition for Determination of Nonregulated Status" to the USDA, APHIS for two lepidopteran-insect resistant cotton and bromoxynil tolerant events, designated 31807 and 31808, that are defined as those cotton plants that express *cryIA(c)* gene coding for CryIA*(c)* toxin protein from the *Bacillus thuringiensis* subsp. *kurstaki* (*Btk)* that confers insect resistance to lepidopteran insects and the bromoxynil-specific nitrilase from *Klebsiella pneumoniae*. Another gene (*kan*^{R}*)* that is used to select for the transgenic plants in the laboratory has been introduced into these plants. This marker gene, from *Escherichia coli*, encodes the enzyme, neomycin phosphotransferase, that inactivates the antibiotic kanamycin (and its chemical relatives). Two promoters were used to direct the expression of these genes in the plant. Promoters direct the production of the proteins to the cells where the gene product must be produced so the transgenic plants express the desired phenotype. One promoter was derived from cauliflower mosaic virus and is called the

35S promoter and the second was a chimera of the 35S promoter and the promoter from a mannopine synthase gene from *Agrobacterium tumefaciens*. These genes were introduced into these cotton plants 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. The potential commercial use of these plants may offer farmers additional choices for the control lepidopteran insects and for weed control.

B. APHIS Regulatory Authority. APHIS regulations at 7 CFR Part 340, which were promulgated pursuant to authority granted by the Federal Plant Pest Act, (7 U.S.C. 150aa-150jj) as amended, and the Plant Quarantine Act, (7 U.S.C. 151-164a, 166-167) as amended, regulate the introduction (importation, interstate movement, or release into the environment) of certain genetically engineered organisms and products. 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. The bromoxynil tolerant and lepidopteran insect resistant cotton plants described in the petition have been considered regulated articles because they contain noncoding DNA regulatory sequences derived from plant pathogens, and because portions of the plasmid vector are derived from plant pathogens, and the vector agent used to deliver the plasmid vector is a plant pathogen.

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 therefore should no longer be regulated. If APHIS determines that the regulated article is unlikely to present a greater plant pest risk than the unmodified organism, the Agency will grant the petition in whole or in part. As such, APHIS permits would no longer be required for field testing, importation, or interstate movement of the nonregulated article or its progeny.

C. EPA and FDA Regulatory Authority.

These genetically engineered cotton plants are also currently 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. 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.

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, including those genetically engineered, was published in the Federal Register on May 29, 1992, and appears at 57 FR 22984-23005.

III. PURPOSE AND NEED

APHIS has prepared this EA before making a determination on the status of these two cotton plants as regulated articles under APHIS regulations. The developer of these cotton plants, Calgene, submitted a petition to USDA, APHIS requesting that APHIS make a determination that these two plants shall no longer be considered regulated articles under 7 CFR Part 340.

This EA was prepared in compliance with: (1) The National Environmental Policy Act of 1969, as amended (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).

IV. ALTERNATIVES

A. No Action.

Under the Federal "no action" alternative, APHIS would not come to a determination that these plants are not regulated articles under the regulations at 7 CFR Part 340. Permits issued or notifications acknowledged by APHIS would still be required for introductions of these plants. APHIS might choose this alternative if there were insufficient evidence to demonstrate the lack of plant pest risk from uncontained cultivation of these plant plants.

B. Determination that Events 31807 and 31808 Are No Longer Regulated Articles.

Under this alternative, these two plants would no longer be regulated articles under the regulations at 7 CFR Part 340. Permits issued or notifications acknowledged by APHIS would no longer be required for introductions of these plants. A basis for this determination would include a "Finding of No Significant Impact" under the National

Environmental Policy Act of 1969 (42 USC 4331 *et seq.*; 40 CFR 1500-1508; 7 CFR Part 1b; 7 CFR Part 342).

V. POTENTIAL ENVIRONMENTAL IMPACTS

Potential impacts to be addressed in this EA are those that pertain to the use of events 31807 and 31808 cotton in the absence of confinement.

Potential impacts from pollen escape and outcrossing of Events 31807 and 31808 cotton with wild relatives

None of the relatives of cotton found in the United States (*G. barbadense*, *G. thurberi*, and *G. tomentosum*) show any definite 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*.

Events 31807 and 31808 cotton is chromosomally compatible with wild *G. hirsutum*. However, according to Dr. Paul Fryxell of Texas A & M University, a leading authority on the systematics and distribution of Gossypieae, 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* found around the Gulf of Mexico 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 is 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. 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 would not significantly 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 events 31807 and 31808 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.

Even if the new traits do introgress into *G. barbadense*, the added traits will confer minimal selective advantage in the wild species (see below).

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 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 plants bred by traditional means, which should be no more or less likely to interbreed with *G. tomentosum* than events 31807 and 31808 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 events 31807 and 31808 cotton, based on the facts that: the transgenic variety poses no increased weediness itself; the two species are unlikely to successfully cross in nature; and the added traits will confer minimal selective advantage in the wild species habitat (see below).

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 where *G. hirsutum* and *G. barbadense* grow together in Central America or the Caribbean. 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 events 31807 and 31808 cotton to cultivated or occasional noncultivated *G. barbadense* would therefore not likely occur at a high level. Any movement of genetic material from events 31807 and 31808 cottons into *G. barbadense* is likely to be the result of intentional breeding practice rather than accidental crossing.

Potential impacts based on increased weediness of Events 31807 and 31808 cotton relative to traditionally bred cotton

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) 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 is rare and anecdotal, and vague as to the nature of the problem.

The relevant introduced traits, bromoxynil tolerance and lepidopteran insect resistance, are unlikely to increase weediness of this cotton or any sexually compatible species present in the U.S. To increase weediness of the cotton plant there would have to be selection pressure (Tiedje et al., 1989; Office of Technology Assessment, 1988). In agricultural settings, currently available herbicide tolerant cotton plants are no more difficult to control (i.e., weedier) than nontolerant plants. Even in the unlikely event of the movement of a bromoxynil tolerance gene to other *Gossypium* species (see above) found in the U.S., bromoxynil is not currently used on these plants as a control measure. Even if such bromoxynil-resistant *Gossypium* plants did exist, many other methods of control would be readily available to eliminate these plants.

Gossypium species are susceptible to many pests and diseases including: bollworms, budworms, armyworms, cabbage loopers, caterpillars, boll weevil, nematodes, mites, seedling diseases, *Verticillium*, *Fusarium*, *Xanthomonas*, *Puccinia*, and *Alterneria* (Anonymous, 1984). Although the budworm and bollworm insects that will be controlled by *cryIA(c)* gene are serious pests of cotton and elimination of these pests may result in increase growth of the plant, significant number of other pests (e.g., boll weevils) and disease pressures (e.g., seedling pathogens and bollrots) from these other organisms are still present to affect the plants. Because events 31807 and 31808 cotton are expected to be cultivated like any other cotton in a managed agricultural ecosystem, the likelihood that sufficient selective pressure would be present for these two cotton plants to become a weed is low.

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 events 31807 and 31808 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. With respect to these issues, APHIS would like to note that a Rhone-Poulenc Ag Company which manufactures bromoxynil has filed (61 FR 67807-67811, December 24, 1996) with the EPA a pesticide petition for the use of bromoxynil on bromoxynil tolerant cotton plants developed by Calgene. Previously, APHIS (petition 93-196-01p) granted nonregulatory status to several bromoxynil tolerant cotton developed by Calgene. In addition, the Monsanto Agricultural Company, the parent company of Calgene, has been granted an exemption of tolerance for CryIA(c) for cotton (60 FR 47871-47874, September 15, 1995). The *cryIA(c)* gene used in these transformants differs mainly in that the gene is truncated which is unlikely to alter its biological activity. Calgene has requested that its *cryIA(c)* gene be considered by the EPA as an amended formulation of the Monsanto registration. Previously, APHIS (petition 94-308-01p) granted nonregulatory status to several lepidopteran insect resistant cotton plants using CryIA(c) developed by Monsanto.

CryIA*(c)*, expressed in lepidopteran-resistant cotton plants, 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. 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 events 31807 and 31808 cotton. The novel proteins that will be expressed in these cotton plants are not known to have any toxic properties on 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 these cotton plants 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) proteins 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 neomycin phosphotransferase conferring kanamycin resistance used as a selectable marker during development of transgenic cotton plants and the nitrilase enzyme used to encode tolerance to the herbicide bromoxynil. The narrow range of substances that can act as substrates for these two enzymes suggests that it is unlikely that they would act on any endogenous substance in any organism that might eat bromoxynil tolerant cotton in the field to produce novel compounds toxic to it. 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.

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. A concern is whether bollworm and budworm will develop resistance to the CryIA(c) protein. EPA has recognized that value of *Bt* as a safer pesticide and has determined that it is necessary to conserve this resource as appropriate by requiring resistance management plans. This has included implementation of a management plan for Monsanto's lepidopteran resistant cotton expressing CryIA(c). Since Calgene has requested registration of the *cryIA(c)* genes used in events 31807 and 31808 under Monsanto's registration, these transgenic plants will likely be used under an approved resistance management plan. 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.

APHIS has identified no factors that would suggest any likelihood of impacts to the environment outside the continental United States. While isolated environments, such as are found in Hawaii or in territories or possessions of the United States, have fragile ecologies that have frequently been damaged through human intervention, APHIS has determined that events 31807 and 31808 cotton will have impacts no different from traditional cotton varieties that are not subject to petition requirements under 7 CFR Part 340 before they enter agriculture. Accordingly, option (A) is rejected. Therefore, option (B) is adopted.

VI. SUMMARY

In accordance with the requirements of NEPA, APHIS has considered the potential for significant impact on the environment of a proposed action, i.e., reaching the determination that events 31807 and 31808 cotton, that have been field tested under notification prior to submission of petition 97-013-01p to APHIS, have no potential to pose a plant pest risk and should no longer be considered regulated articles under the regulations at 7 CFR Part 340. After careful analysis of the available information, APHIS concludes that its proposed action should present no significant impact on the environment. This conclusion is based on factors discussed herein or in the determination included as an appendix, as well as the following factors:

1. Genes that confer tolerance to the herbicide bromoxynil, resistance to lepidopteran insects, and a marker gene that confers resistance to the antibiotic kanamycin have been inserted into a cotton chromosome in cotton plants denoted events 31807 and 31808 cotton. Neither the genes that confer bromoxynil herbicide tolerance, kanamycin resistance, lepidopteran insect resistance, the products of the genes, nor their associated regulatory sequences derived from plant pests, confer on events 31807 and 31808 cotton any plant pest characteristic.

2. In nature, the genes that confer tolerance to the herbicide bromoxynil and lepidopteran insect resistance will not provide the events 31807 and 31808 cotton plants with any significant selective advantage over nontransformed cotton plants in their ability to disseminate or to become established in the environment. There is no reason to believe that events 31807 and 31808 cotton exhibit any increased weediness relative to that of traditional varieties.

3. In nature, chromosomal genetic material from plants can only be transferred to another sexually compatible flowering plant by cross-pollination. The sexually compatible species in the United States are *Gossypium hirsutum* (i.e., other cultivated cotton or wild cotton), *G. tomentosum*, and *G. barbadense*. There is no reason to believe that the use of events 31807 and 31808 cotton in agriculture will lead to an increase in weediness in any plant with which it can successfully interbreed.

4. The use of events 31807 and 31808 cotton or products derived from them will not cause damage to raw or processed agricultural commodities.

5. There is no reason to believe that the use of events 31807 and 31808 cotton in agriculture will have a significant impact on any beneficial organisms in the environment, or on any threatened or endangered species.

6. Even if the target insects develop resistance to the insecticide, this should not reduce the ability to control these insects in cotton or other crops when cultivated because the currrently available insecticides used to control these insects will still be available.

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RESPONSE TO CALGENE PETITION 97-013-01p FOR DETERMINATION OF NONREGULATED STATUS FOR LEPIDOPTERAN RESISTANT AND BROMOXYNIL TOLERANT EVENTS 31807 AND 31808 COTTON

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United States Department of Agriculture
Animal and Plant Health Inspection Service
Plant Protection and Quarantine

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I. SUMMARY

This Animal and Plant Health Inspection Service (APHIS) determination has been made in response to a petition received on January 13, 1997 from Calgene, Inc., of Davis, California. The petition seeks a determination from APHIS that events 31807 and 31808 cotton do not present a plant pest risk and are therefore no longer regulated articles. On February 21, 1997, APHIS announced receipt of the Calgene petition in the Federal Register and stated that the petition was available for public view. Based on a review of scientific data, APHIS has determined events 31807 and 31808 cotton do not present a plant pest risk and are therefore no longer regulated articles under its regulations at 7 CFR Part 340.

Calgene has submitted a "Petition for Determination of Nonregulated Status" to the USDA, APHIS for two lepidopteran-insect resistant cotton and bromoxynil tolerant events, designated 31807 and 31808, that are defined as those transgenic cotton plants that express a truncated *cryIA(c)* gene coding for insecticidal protein from the *Bacillus thuringiensis* subsp. *kurstaki* that confers insect resistance to lepidopteran insects and the bromoxynil-specific nitrilase from *Klebsiella pneumoniae*. Another gene (*kan^R*), from *Escherichia coli*, that is used to select for the transgenic plants in the laboratory has been introduced into these two events. This marker gene encodes the enzyme neomycin phosphotransferase that inactivates the antibiotic kanamycin (and its chemical relatives). Two promoters were used to direct the expression of these genes in the plant. Promoters direct the production of the proteins to the cells where the gene product must be made to ensure the plant expresses the intended phenotypes. One of the promoters is derived from cauliflower mosaic virus and is called the 35S promoter and the second is a chimera of the 35S promoter and the promoter from a mannopine synthase gene from *Agrobacterium tumefaciens*. These genes were introduced into these cotton transformants 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

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 will be granted in whole or in part. This allows for unregulated introduction of the regulated article in question.

Events 31807 and 31808 cotton have been field tested under nine APHIS notifications in States. All field trials were performed essentially under conditions of reproductive confinement.

Events 31807 and 31808 cotton contain components from organisms that are known plant pathogens, i.e., the bacterium *Agrobacterium tumefaciens* and cauliflower mosaic virus. These transgenic cotton plants have therefore been a regulated article under APHIS jurisdiction. An APHIS determination that events 31807 and 31808 cotton do not present a plant pest risk is based on an analysis of data provided to APHIS by Calgene and other relevant published scientific data obtained by APHIS concerning the components of lepidopteran-resistant and bromoxynil tolerant cotton, observable properties of the cotton plants themselves. From this review, we have determined that events 31807 and 31808: (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; (5) are not likely to harm other organisms, such as bees and earthworms, that are beneficial to agriculture; and (6) should not reduce the ability to control insects in cotton or other crops when cultivated. In addition, we have determined that new progeny cotton lines bred from these two events will not exhibit new plant pest properties, i.e., properties substantially different from any observed for insect resistant and herbicide tolerant cotton plants already field tested, or those observed for cotton in traditional breeding programs.

Calgene has provided general information and data from field testing of events 31807 and 31808 cotton. The effect of this determination is that these two events will no longer be considered regulated articles under APHIS regulations at 7 CFR Part 340. Notifications under those regulations will no longer be required from APHIS for field testing, importation, or interstate movement of these two events or their progeny. Importation of these two events 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.

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, as amended (42 U.S.C. 4321 *et seq.*; 40 CFR 1500-1508; 7 CFR Part 1b; 7 CFR part 372). An environmental assessment (EA) was prepared and a finding of no significant impact (FONSI) was reached by APHIS for the determination that events 31807 and 31808 cotton are no longer regulated articles 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/biotech.

II. BACKGROUND

USDA Regulatory Framework. APHIS regulations found at 7 CFR Part 340 (hereafter referred to as the regulations) were promulgated pursuant to authority granted by the Federal Plant Pest Act (FPPA)(7 U.S.C. 150aa-150jj) as amended, and the Plant Quarantine Act (PQA), (7 U.S.C. 151-164a, 166-167) as amended. The regulations pertain to the introduction (importation, interstate movement, or release into the environment) of certain genetically engineered organisms and products.

Events 31807 and 31808 cotton 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 three genes 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.

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

Oversight by Other Federal Agencies. APHIS' decision on the regulatory status of the lepidopteran-resistant and bromoxynil tolerant cotton, under APHIS' regulations at 7 CFR 340, in no way releases these cotton plants from EPA and FDA regulatory oversight.

III. PUBLIC COMMENTS

No comments were received on this petition by the close of the comment period on April 22, 1997.

V. BIOLOGY OF 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 events 31807 and 31808 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 are found 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).

V. RATIONALE FOR THE DEVELOPMENT OF EVENTS 31807 AND 31808 COTTON

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.

Current methods for weed control in cotton production are cultural practices (e.g., cultivar selection, seedbed preparation), mechanical tillage, and chemical control. Most cotton today is grown using herbicides: 88 percent of upland cotton acreage in the United States in 1992 received herbicide treatments (USDA, 1993). In 1990, cotton farmers applied, on average, 2.1 herbicide treatments per acre per growing season (USDA, 1991). Herbicides used in cotton cultivation may be applied in a variety of preplant, preemergence, or postemergence treatments. Some of the herbicides currently used in cotton cultivation are trifluralin, fluometuron, prometryn, and mono- and disodium methylarsonate. Continuous repeated use of the same herbicide over many growing seasons has been implicated in declining cotton yields (Frans et al., 1982; Rogers et al., 1983; Talbert et al., 1983). There is increasing commercial interest over the past few years in the "organic" cultivation of cotton.

It has been projected that without herbicide use, cotton production would be reduced by approximately 32 percent (Abernathy, 1981). It was estimated that weed interference accounted for cotton production losses of 8.4 percent in 1983, even with herbicide use (Whitwell and Everest, 1984).

VI. PLANT PEST RISK ANALYSIS OF EVENTS 31807AND 31808 COTTON

To reach the determination that events 31807 and 31808 cotton does not present a plant pest risk, APHIS analyzed public comments, basic information on the biology of cotton, data presented by Calgene, and other relevant scientific data pertaining to the petition. Based on the data described, APHIS has arrived at a series of conclusions:

A. The introduced genes, their regulatory sequences and their products do not present a plant pest risk.

Events 31807 and 31808 cotton were produced using an *Agrobacterium*-mediated transformation protocol. In this transformation system, all the genes involved in pathogenicity of *Agrobacterium* have been removed. The cotton line Coker 130 was transformed with a plasmid containing the CryIA(c) which confers resistance to some lepidoptera, the bromoxynil-specific nitrilase which encodes herbicide tolerance, 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 modified from the original sequence found in the bacterium in two ways. First, only amino acids 25 to 613 were used in engineering the two cotton events. δ-endotoxins are large proteins that upon ingestion by the insect is cleaved by proteases into smaller peptides. The toxic moieties are the smaller fragments. Thus, the truncated CryIA(c) inserted into the plant will still be further cleaved into the insecticidal toxic fragments. 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. Second, the nucleotide sequence was modified for plant codon preferences. This modification allows for more protein to be produced in the plant and does not significantly alter the final protein product or biological activity. The expression of the *cryIA(c)* gene was under the MAC promoter which is a hybrid of the 35S promoter from cauliflower mosaic virus (CaMV) and the mannopine synthase promoter from *A. tumefaciens*. The termination sequence for this gene was derived from mannopine synthase enzyme from *A. tumefaciens*.

The bromoxynil-specific nitrilase gene was isolated from a strain of the bacterium *Klebsiella pneumoniae* subsp. *ozaenae*. This species is a soil microorganism which is not known to cause disease in animals or plants. The enzyme nitrilase catalyzes a specific chemical reaction, namely the breakdown of the herbicide bromoxynil (3,5-dibromo-4-hydroxybenzonitrile) to 3,5-dibromo-4-hydroxybenzoic acid. Production of the nitrilase enzyme is directed by 35S promoter from CaMV. The termination sequence was isolated from *tml3'* gene from *A. tumefaciens*.

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) and nitrilase genes. 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 these two cotton plants, and its presence does not imply that kanamycin will be used in the cultivation of cotton. The synthesis of nptII in the plant was directed by the 35S CaMV promoter and termination sequences from *tml3'* gene from *A. tumefaciens*. Calgene has provided Southern data to show that these and only these genes are present in events 31807 and 31808. Calgene has provided data to demonstrate that no sequences from outside the border region of the vector system are present.

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.

B. Events 31807 and 31808 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; from this core, individual definitions differ in approach and emphasis. 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.

The introduction of the insect resistance phenotype into these two cotton plants will not significantly increase their weediness potential. *Gossypium* species are susceptible to many pests and diseases including: bollworms, budworms, armyworms, cabbage loopers, caterpillars, boll weevil, nematodes, mites, seedling diseases (*Phythium*, *Rhizoctonia*, and *Thielaviopsis*), *Verticillium*, *Fusarium*, *Phymatotrichum*, *Xanthomonas*, *Puccinia*, and *Alterneria* (Anonymous, 1984). Although the budworm and bollworm that will be controlled by *cryIA(c)* gene are serious pests of cotton and elimination of theses pests may result in increased growth of the plant, significant number of other pests (e.g., boll weevils) and disease pressures (e.g., seedling pathogens and bollrots) from these other organisms that are still present to affect the plants. Because events 31807 and 31808 cotton are expected to be cultivated like any other cotton in a managed agricultural ecosystem, the likelihood that sufficient selective pressure would be present for these transgenic plants to become a weed is low.

The introduction of the herbicide tolerance phenotype into these two cotton plants will not significantly increase their weediness potential. In agricultural settings, currently available herbicide tolerant cotton plants are no more difficult to control (i.e., weedier) than nontolerant plants. Even in the unlikely event of the movement of a bromoxynil tolerance gene to other *Gossypium* species (see above) found in the U.S., bromoxynil is not currently used on these plants as a control measure. Even if such bromoxynil-resistant *Gossypium* plants did exist, many other methods of control would be readily available to eliminate these plants.

C. Events 31807 and 31808 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 these transgenic cotton plants 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. Even if the new traits do introgress into *G. barbadense*, the added traits will confer minimal selective advantage in the wild species (see below).

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 these transgenic cotton plants 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 these transgenic cotton plants 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, hybrids from crosses from these two species are rare or absent in areas in 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 genetic material from these transgenic cotton plants to cultivated or occasional noncultivated *G. barbadense* would therefore not likely to occur at a high level. Any movement of genetic material from these transgenic cotton plants into *G. barbadense* is likely to be the result of intentional breeding practice rather than accidental crossing.

D. Events 31807 and 31808 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). Calgene has demonstrated several agronomic characteristics including, fiber quality, seed germination, and yield, do not significantly differ from events 31807 and 31808 cotton (or their progeny) versus other nontransgenic cotton lines.

E. Events 31807 and 31808 cotton will not be harmful to beneficial organisms, including bees.

Consistent with its statutory authority and requirements under NEPA, APHIS evaluated the potential for events 31807 and 31808 cotton plants, plant products, and the CryIA(c) insect control protein, and the bromoxynil-specific nitrilase 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 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.

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).

Calgene provided data to demonstrate that the events 31807 and 31808 cotton 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.

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.

In a previous petition (93-196-01p), Calgene provided data to show that the introduced nitrilase has a high specificity for bromoxynil. The high specificity of this enzyme makes it unlikely that the nitrilase would metabolize endogenous plant substrates to produce compounds toxic to beneficial or threatened and endangered species.

APHIS concludes that events 31807 and 31808 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

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 have 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.

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. A concern is whether bollworm and budworm will develop resistance to the CryIA(c) protein. EPA has recognized that value of *Bt* as a safer pesticide and has determined that it is necessary to conserve this resource as appropriate by requiring resistance management plans. This has included implementation of a management plan for Monsanto's lepidopteran resistant cotton expressing CryIA(c). Since Calgene has requested registration of the *cryIA(c)* genes used in events 31807 and 31808 under

Monsanto's registration, these transgenic plants will likely be used under the previously approved resistance management plan. 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.

VII. CONCLUSION

APHIS has determined that events 31807 and 31808 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 these two events or their progeny. Importation of this 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 these transgenic cottons: (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; (5) are not likely to harm other organisms, such as bees and earthworms, that are beneficial to agriculture; and (6) should not reduce the ability to control insects in cotton or other crops when cultivated. APHIS has also concluded that new cotton varieties bred from these events will not exhibit new plant pest properties, i.e., properties substantially different from any observed for the lepidopteran-resistant and bromoxynil tolerant cotton already field tested, or those observed for cotton in traditional breeding programs.

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