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

DEPARTMENT OF AGRICULTURE

Animal and Plant Health Inspection Service

[Docket No. 93-116-2]

Availability of Determination of Nonregulated Status of Calgene, Inc., Genetically Engineered Cotton Lines

AGENCY: Animal and Plant Health Inspection Service, USDA.

ACTION: Notice of determination.

SUMMARY: The Animal and Plant Health Inspection Service (APHIS) is announcing the issuance of a determination that certain trademarked cotton lines, designated BXN™ cotton, do not present a plant pest risk and are therefore no longer regulated articles under its regulations. APHIS' determination has been made in response to a petition received from Calgene, Inc., of Davis, CA, on July 15, 1993, seeking a determination from APHIS that BXN™ cotton does not present a plant pest risk and is therefore no longer a regulated article. The effect of this determination is that cotton lines meeting the definition of BXN™ cotton and that have been field tested under permit, will no longer be subject to regulation. This notice also announces the availability of the determination that provides the basis for the ruling, as well as the availability of an environmental assessment of this action.

EFFECTIVE DATE: February 15, 1994.

ADDRESSES: The determination, the environmental assessment, the Calgene, Inc. submission, and written comments received in response to our September 8, 1993, notice published in the Federal Register 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 access to this room are requested to call ahead on (202) 690-2817.

FOR FURTHER INFORMATION CONTACT: Dr. Michael Schechtman, Senior Microbiologist, Biotechnology, Biologics, and Environmental Protection, APHIS, USDA, room 850 Federal Building, 6505 Belcrest Road, Hyattsville, MD 20782, (301) 436-7601. For a copy of the determination or the environmental assessment, please write or call Ms. Kay Peterson at this same address and telephone number.

SUPPLEMENTARY INFORMATION: On September 8, 1993 (58 FR 47249-47250, Docket No. 93-116-1), the Animal and Plant Health Inspection Service (APHIS) published a notice announcing receipt of a petition from Calgene, Inc. (Calgene) of Davis, CA, that requested a determination on the regulatory status of BXN™ cotton. This notice also indicated the role of the Food and Drug Administration and the United States Environmental Protection Agency in the regulation of food products derived from BXN™ cotton and the potential use of the herbicide bromoxynil on BXN™ cotton, respectively. This notice further announced that the petition was available for public review and invited written comments on whether BXN™ cotton poses a plant pest risk, to be submitted on or before November 8, 1993.

Comments

APHIS received a total of 45 comments from State officials, universities, farmers associations and cooperative extension services, environmental and consumer organizations, and business and professional associations. Among these commenters, 34 were in favor of granting the petition, 9 were opposed, and 2 others addressed APHIS' decision on the petition itself only parenthetically. APHIS has provided a complete discussion of the comments and any issues raised by the commenters in the determination document, which is available upon request from the individual listed under FOR FURTHER INFORMATION CONTACT.

BXN™ cotton, as defined by its developer (Calgene, Inc., of Davis, CA), is any cotton cultivar or progeny of a cotton line containing the BXN gene (a gene, derived from the soil microbe *Klebsiella pneumoniae* subsp. *ozaenae* that encodes the enzyme nitrilase, which can degrade the herbicide bromoxynil) with its associated regulatory sequences, i.e., sequences that allow for expression of the gene's enzyme product. By definition, BXN™ cotton may also contain: the *kanr* marker gene (encoding the enzyme aminoglycoside 3'-phosphotransferase II, which confers resistance to the antibiotic kanamycin) with its associated regulatory sequences; a DNA fragment containing the origin of replication of the pRi plasmid from *Agrobacterium rhizogenes*; T-DNA left and right border sequences from an *Agrobacterium tumefaciens* Ti plasmid; a segment of DNA from transposon Tn5; a portion of a synthetic polylinker sequence from *lacZ'*; and a segment of DNA containing the origin of replication of plasmid pBR322. Expression of the BXN™ gene and the *kanr* gene is directed by copies of the promoter from the *35S* gene from cauliflower mosaic virus and terminated using sequences derived from the *tml* gene from the octopine-type Ti plasmid pTiA6 from *A. tumefaciens*.

BXN™ cotton contains components from organisms that are known plant pathogens, i.e., the bacterium *Agrobacterium tumefaciens* and cauliflower mosaic virus. BXN™ cotton has therefore been a regulated article under APHIS jurisdiction, and its field tests in 1989, 1990, 1991, 1992, and 1993 have been in accordance with APHIS regulations at 7 CFR part 340. APHIS' determination that BXN™ cotton that has been field tested under permit does 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 BXN™ cotton and observable properties of the cotton lines themselves. From this review, we have determined that these BXN™ cotton lines: (1) Exhibit no plant pathogenic properties; (2) are no more likely to become a weed than their non-engineered parental varieties; (3) are unlikely to increase the weediness potential for any other cultivated plant or native wild species with which the organism can interbreed; (4) will not cause damage to processed agricultural commodities; and (5) are unlikely 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 progeny BXN™ cotton lines bred from these lines will not exhibit new plant pest properties, i.e., properties substantially different from any observed for the BXN™ cotton lines already field tested, or those observed for cotton in traditional breeding programs. However, APHIS believes that it is prudent to require information to corroborate that new BXN™ cotton lines, not derived from BXN™ lines already field tested under permit, do not exhibit unexpected qualities.

Calgene has provided information and data from field testing of some of the cotton lines fitting their definition of BXN™ cotton and intended to be representative of all those lines. Our determination, however, applies only to cotton lines that fit Calgene's definition of BXN™ cotton and that have been field tested under permit. The effect of this determination is that such cotton lines will no longer be considered regulated articles under the 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 BXN™ cotton lines that have been field tested under permit or their progeny. Normal agronomic practices involving these BXN™ cotton lines, e.g., cultivation, propagation, movement, and cross-breeding with other non-regulated cotton lines, can now be conducted without an APHIS permit. (Importation of BXN™ cotton (and nursery stock or seeds capable of propagation) is still, however, subject to the restrictions found in the Foreign Quarantine Notices regulations at 7 CFR part 319.) Variety registration and/or seed certification for individual cotton lines carrying the BXN™ 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 parts 1500–1508; 7 CFR part 1b; 44 FR 50381–50384; and 44 FR 51272–51274). An Environmental Assessment (EA) was prepared and a Finding of No Significant Impact (FONSI) was reached by APHIS for the determination that BXN™ cotton that has been field tested under permit is no longer a regulated article under its regulations at 7 CFR part 340.

Done in Washington, DC, this 15th day of February 1994.

Lonnie J. King,

Acting Administrator, Animal and Plant Health Inspection Service.

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United States Department of Agriculture

Animal and Plant Health Inspection Service

USDA/APHIS Petition P93-196-01 for Determination That BXN™ Cotton Poses No Plant Pest Risk

Environmental Assessment February 1994

Finding of No Significant Impact (FONSI) for Nonregulatory Status of BXN™ Cotton, Calgene Petition P93-196-01

The Animal and Plant Health Inspection Service (APHIS) of the U.S. Department of Agriculture has conducted an environmental assessment in response to a petition (APHIS Number P93-196-01) received from Calgene, Inc., regarding the status of trademarked cotton lines, designated BXN™ cotton, under APHIS regulations at 7 CFR Part 340. The plants have been engineered with a selectable genetic marker gene and a gene that confers tolerance to the herbicide bromoxynil. Based upon the analysis documented in its environmental assessment, APHIS has reached a finding of no significant impact on the environment from its determination that cotton lines fitting the definition of BXN™ cotton that have been field tested prior to the submission of the petition will no longer be regulated articles.

John H. Payne, Ph.D.
Acting Director
Biotechnology, Biologics, and Environmental Protection
Animal and Plant Health Inspection Service
U.S. Department of Agriculture

Date: FEB 15 1994

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I. Summary

The U.S. Department of Agriculture, Animal and Plant Health Inspection Service (APHIS) has prepared an environmental assessment in response to a petition (APHIS Number P93-196-01) from Calgene, Inc., regarding trademarked lines of cotton (*Gossypium hirsutum*) designated "BXN™ cotton." Calgene seeks a determination from APHIS that BXN™ cotton does not present a plant pest risk and is therefore no longer a regulated article. The significant modification to the BXN™ cotton plants relative to traditional cotton varieties is that BXN™ cotton has been modified to express one genetic marker gene and another gene that provides tolerance to herbicide bromoxynil.

APHIS regulations at 7 CFR Part 340, which 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, 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, thereby allowing for unregulated introduction of the article in question. Permits under those regulations will no longer be required from APHIS for field testing, importation, or interstate movement of that article or its progeny. Normal agronomic practices with it, e.g., cultivation, propagation, movement, and cross-breeding could then be conducted without APHIS permit.

BXN™ cotton lines have previously been field tested under 15 APHIS permits. Permitted field tests took place at a total of 57 sites in 13 states. Field tests were also conducted in Argentina, Bolivia, and South Africa in accordance with national regulatory requirements. Additional demonstration trials using BXN™ cotton were also performed under notification during the growing season just ended. All field trials were performed essentially under conditions of reproductive confinement.

APHIS has considered the information provided by Calgene in its petition as well as other scientific data and comments received from the public relating to the potential plant pest risk of BXN™ cotton. APHIS has made the determination that BXN™ cotton has no potential to pose a plant pest risk, and determined that BXN™ cotton lines that have been previously field tested under permit are no longer regulated articles. Our documentation of that determination is attached as an appendix. In reaching that determination, in accordance with regulations and guidelines implementing the National Environmental Policy Act of 1969 (42 U.S.C. 4321 *et seq.*; 40 CFR 1500-1508; 7 CFR Part 1b; 44 FR 50381-50384; and 44 FR 51272-51274), APHIS has evaluated

the potential for significant impact to the human environment from its determination. That analysis is presented herein.

An environmental assessment (EA) was prepared prior to granting each of the permits for a field trial using BXN™ cotton. The 15 previous EA's discussing BXN™ cotton addressed issues pertinent to plant pest risk issues relative to the conduct of field trials under reproductive confinement. The technical discussion of plant pest risk issues is reiterated and expanded in our determination in the appendix. The analyses in the 15 previous EA's, though adequate to address most of the issues relating to the properties of BXN™ cotton, do not address several new issues that are of relevance to the growth of BXN™ cotton in the absence of such confinement. The main new issues that are considered in this EA are:

* Is there any potential for significant impact to the environment based on increased weediness of BXN™ cotton relative to traditionally bred cotton?

* Will outcrossing of BXN™ cotton with any wild plant relatives result in any significant impact to the environment? and

* Will the use of BXN™ cotton result in any significant impact on any unrelated (nontarget) organisms, including both beneficial organisms such as bees and earthworms as well as endangered species?

Based on its review, APHIS has concluded that: (1) there is no reason to believe that BXN™ cotton should exhibit enhanced weediness relative to traditionally bred cotton, which is not considered to show particular weedy aggressive tendencies; (2) although outcrossing is likely to occur at some level between BXN™ cotton and other wild or cultivated cottons or cotton relatives, there is no reason to believe that outcrossing will either increase the weediness of other cotton populations or otherwise impact them differently from outcrossing with traditionally bred cottons; and (3) there is no reason to believe that BXN™ cotton will have any significant impact on any nontarget species.

This EA specifically addresses the potential for impacts to the human environment through the use in agriculture of BXN™ cotton. It does not address the separate issue of the potential use of the herbicide bromoxynil (Buctril) in conjunction with BXN™ cotton. The U.S. Environmental Protection Agency (EPA) has authority over the use in the environment of all pesticidal substances, including herbicides, under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA); in particular, EPA has jurisdiction over registration of bromoxynil for use on cotton. The potential issuance by EPA of a new label for use of the herbicide bromoxynil on BXN™ cotton and the determination by APHIS regarding the cotton itself are independent decisions, under consideration by different agencies, based on distinct regulations under unrelated legal authorities, in response to requests from two separate corporate entities. We have considered the

potential for impacts outside the boundaries of the United States in response to comments although APHIS' decision only goes to actions within the United States.

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 BXN™ cotton lines that have been previously field tested under the regulations at 7 CFR Part 340 will no longer be regulated articles. The FONSI accompanies this document.

II. Introduction

A petition was submitted to USDA/APHIS pursuant to regulations codified in 7 CFR Part 340 which are entitled "Introduction of Organisms and Products Altered or Produced Through Genetic Engineering Which Are Plant Pests or Which There Is Reason to Believe Are Plant Pests." The regulations govern the introduction (importation, interstate movement, or release into the environment) of certain genetically engineered organisms and products. Under section 340.0 of the regulations, a person is required to obtain a permit prior to introducing a regulated article. Prior to issuing a permit, APHIS conducts an analysis of the potential impacts associated with the proposed introduction, and publishes an environmental assessment which documents the analysis in accordance with regulations and guidelines implementing the National Environmental Policy Act of 1969 (42 U.S.C. 4321 *et seq.*; 40 CFR 1500-1508; 7 CFR Part 1b; 44 FR 50381-50384; and 44 FR 51272-51274). Certain field trials may be conducted according to the requirements of a notification option described under section 340.3. (A Special Assessment relative to the introduction of regulated articles under notification was prepared by APHIS prior to promulgation of the notification option. It is available upon request.)

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 transgenic cotton plants described in the Calgene petition have been considered regulated articles because noncoding DNA regulatory sequences and portions of the plasmid vector are derived from plant pathogens.

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. Among other data requirements, section 340.6 (c)(3) requires that a petitioner,

> ". . . Describe known and potential differences from the unmodified organism that would substantiate that the regulated article is unlikely to pose a greater plant pest risk than the unmodified organism from which it was derived, including but not limited to: plant pest risk characteristics, disease and pest susceptibilities, expression of the gene product, new enzymes, or changes to plant metabolism, weediness of the regulated article, impact on the weediness of any other plant with which it can interbreed, agricultural or cultivation practices, effects of the regulated article on nontarget organisms, indirect plant pest effects on other agricultural products, transfer of genetic information to organisms with which it cannot interbreed, and any other information which the Director believes to be relevant to a determination. Any information known to the petitioner that a regulated article may pose a greater plant pest risk than the unmodified recipient organism shall also be included."

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, thereby allowing for unregulated introduction of the article in question.

III. Alternatives

APHIS has considered the following four alternatives with respect to Calgene's petition:

(1) deny the petition, so that BXN™ cotton plants would continue to be regulated articles under 7 CFR Part 340;

(2) approve the petition for all cotton lines fitting the definition of BXN™ cotton, regardless of whether they have been field tested or produced in the laboratory to date;

(3) approve the petition for all cotton lines fitting the definition of BXN™ cotton that have been field tested prior to the submission of the subject petition; or

(4) approve either alternative (2) or (3) with the additional stipulation that the determination applies to the use of BXN™ cotton within the continental United States.

IV. Scope

This EA is concerned with potential environmental impacts from the unrestricted introduction of BXN™ cotton. The genetic material introduced into BXN™ cotton has been discussed in detail in EA's prepared for 15 prior field tests under APHIS permits 92-106-01, 92-105-01, 91-357-01, 91-333-02, 91-329-04, 91-329-03, 91-329-02, 91-329-01, 91-107-06, 91-035-07, 90-303-02, 90-297-01, 90-016-04, 89-192-01, and 89-047-07. Permitted field tests took place at a total of 57 sites in the following 13 states: Alabama, Arizona, Arkansas, California, Georgia, Hawai'i, Louisiana, Mississippi, Missouri, North Carolina, South Carolina, Tennessee, and Texas. Field tests were also conducted in Argentina, Bolivia, and South Africa in accordance with national regulatory requirements. Additional demonstration trials using BXN™ cotton were also performed under notification during the growing season just ended. All field trials were performed essentially under conditions of reproductive confinement. Further discussions of the biology of cotton as well as of the genetic components used to construct BXN™ cotton are found in APHIS' Determination that BXN™ cotton has no potential to pose a plant pest risk. As this information is included in the appendix, it will not be described in detail in the body of this document.

There are minor differences in the description of cotton varieties tested under the cited permits and the description, in Calgene's petition, of BXN™ cotton, based on new information provided by Calgene concerning the genetic composition of BXN™ cotton transformants. Additional sequences, derived from the region outside the T-DNA borders in the vectors used for the genetic transformation of cotton, may be present in some BXN™ cotton lines. Although some of these sequences may be derived from plant pathogens, none of them is involved in the causation of disease and none will be expressed in BXN™ cotton. These sequences are discussed more fully in the appendix.

Effects associated with the potential uses of the herbicide bromoxynil in conjunction with BXN™ cotton are outside the scope of APHIS' analysis. APHIS' determination does not constitute authorization to use bromoxynil on BXN™ cotton. Rather, EPA has the responsibility for ensuring that any uses of herbicide will not cause unreasonable adverse effects on the environment within the context of FIFRA. Approval by EPA of a particular label condition for a pesticide is granted when, under the specified conditions of use, it will not generally cause such effects. EPA considers both human health and safety as well as nontarget effects of both the herbicide and its breakdown products in making a decision on registration of an herbicide. The potential issuance by EPA of a new label for use of the herbicide bromoxynil on BXN™ cotton and the determination by APHIS regarding the cotton itself are separate decisions, under consideration by different agencies based on distinct regulations under unrelated legal authorities in response to requests from two separate corporate entities.

APHIS has also considered potential effects of the cultivation of BXN™ cotton outside the United States. A general consideration of this topic indicates to us that there are

no necessary impacts on cotton diversity occasioned by this determination to allow the cultivation, without permit, of BXN™ cotton in the United States. Even if BXN™ cotton were to be cultivated in agricultural regions around centers of cotton diversity, it seems extremely unlikely that BXN™ cotton would have any effect on wild progenitors of cultivated cotton. The herbicide bromoxynil is generally used only in agricultural contexts rather than on uncultivated land, and there is no reason to believe that the bromoxynil resistance trait would impart any selective advantage to a recipient plant in the absence of bromoxynil application. In any event, 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). Therefore, this topic will not be discussed in any greater detail. We would, however, note these additional facts: (1) crop plants and seeds exported from the United States, whether transgenic or nontransgenic 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 and/or commerce with transgenic cotton are unaffected by our action; and (4) APHIS has no jurisdiction over approval for the use of bromoxynil on cotton plants in foreign nations. Scenarios in which an impact of BXN™ 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.

V. Potential Environmental Impacts

Potential impacts to be addressed in this EA are those that pertain to the use of BXN™ cotton in the absence of confinement.

Potential impacts based on increased weediness of BXN™ 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; 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 relevant introduced trait, bromoxynil tolerance, is unlikely to increase weediness of this cotton. Bromoxynil would not be applied on BXN™ cotton for the purpose of controlling the cotton itself, but rather for controlling unrelated weeds in the field. To increase weediness of the cotton plant there would have to be selection pressure on BXN™ cotton (Tiedje et al., 1989; Office of Technology Assessment, 1988) associated with bromoxynil use on it. Because bromoxynil will not affect the survival of BXN™ cotton and because *G. hirsutum* is not itself weedy, this type of selection pressure does not now and is unlikely ever to exist. Even if such bromoxynil-resistant weedy plants did exist, bromoxynil treatment would not be the control method of choice; many other methods of control would be readily available.

A review of other new traits, besides bromoxynil resistance, that may be present in the transgenic plant shows no likelihood to increase weediness potential. None of these traits is in any way related to characteristics associated with weediness.

No other variation seen in BXN™ lines is indicative of increased weediness. Calgene's 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. Calgene's burial study shows no obvious increase in volunteer plants from buried seeds. In addition, Calgene's field reports show no obvious increase in volunteers from seed, regrowth from stubble, or increase in seed dormancy. Calgene does report on lint characteristics which may suggest a decrease in seed size. If such a decrease does indeed exist, APHIS believes that no competitive advantage affecting weediness would be conferred on the transgenic plants by this change. Calgene has correctly argued, 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.

Potential impacts from pollen escape and outcrossing of BXN™ cotton with wild relatives

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

BXN™ 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 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 U.S. 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. Even if the nonagricultural 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 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 BXN™ 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 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 lines bred by traditional means, which should be no more or less likely to interbreed with *G. tomentosum* than BXN™ 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 BXN™ 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 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. 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 BXN™ cotton to cultivated or occasional noncultivated *G. barbadense* would therefore not likely occur at a high level. Any movement of genetic material from BXN™ 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 in the absence of sustained bromoxynil use.

Should a movement of genetic material take place to these receptive plants and bromoxynil resistance be transferred, no competitive advantage would be conferred, because bromoxynil is not used with these plants when they are found in nonagricultural areas. In agricultural areas, such plants would be controlled by normal agronomic practices. Therefore, the presence of an occasional bromoxynil-tolerant relative of BXN™ cotton should pose no significant impact to the environment.

Potential impact on nontarget organisms, including beneficial organisms such as bees and earthworms

There is no reason to believe that deleterious effects or significant impacts on nontarget organisms, including beneficial organisms, would result from the cultivation of BXN™ cotton. The novel proteins that will be expressed in BXN™ cotton are not known to have any toxic properties. 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. 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 BXN™ cotton in the field to produce novel compounds toxic to it. 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 BXN™ cotton would have any effect on any other threatened or endangered species in the United States.

VI. Consideration of Alternatives

Based on the information Calgene has provided regarding the properties of the BXN™ cotton lines it has developed and other information from other sources, APHIS has identified no significant impact to the environment from issuance of a determination that BXN™ cotton poses no potential for plant pest risk that would justify denying Calgene's petition. Accordingly, alternative (1) is rejected.

Calgene has provided data that substantiates that the properties of cotton lines that fit the definition of BXN™ cotton and that have been developed to date, exhibit a level of variability consistent with that of traditional cotton varieties. It is expected that the progeny of these cotton lines will exhibit similar levels of variability. It is not unlikely that similar properties will be exhibited by new cotton lines that may be developed in the future that fit the definition of BXN™ cotton. However, APHIS believes that it is prudent to require information to corroborate that new cotton lines do not exhibit unexpected properties. (As experience with such organisms increases, such verification may cease to be essential in the future.) Accordingly, alternative (2) is rejected.

APHIS has identified no factors that would suggest any likelihood of impacts to the environment of the United States but outside the continental United States. While isolated environments, such as are found in Hawai'i or in territories or possessions of the United States, have fragile ecologies that have frequently been damaged through human intervention, APHIS has determined that BXN™ 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, alternative (4) is rejected and alternative (3) is adopted.

VII. 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 lines of BXN™ cotton, that have been field tested under permit prior to submission of petition P93-196-01 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 in the appendix, as well as the following factors:

1. A gene that confers tolerance to the herbicide bromoxynil and a marker gene that confers resistance to the antibiotic kanamycin have been inserted into a cotton chromosome in cotton lines denoted BXN™ cotton. 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*.

2. Neither the gene that confers tolerance to the herbicide bromoxynil nor the gene that confers kanamycin resistance, nor the gene product of either gene, nor their associated regulatory sequences, confers on BXN™ cotton any plant pest characteristic.

3. In nature, the gene that confers tolerance to the herbicide bromoxynil will not provide the BXN™ cotton plants with any measurable 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 BXN™ cotton exhibits any increased weediness relative to that of traditional varieties.

4. There is no reason to believe that the use of BXN™ cotton in agriculture will lead to an increase in weediness in any plant with which it can successfully interbreed.

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

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Appendix

Response to Calgene Petition P93-196-01 for Determination of Nonregulated Status of BXN™ Cotton

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

I. Executive Summary

The Animal and Plant Health Inspection Service (APHIS) has determined, based on a review of scientific data, that trademarked cotton lines, designated BXN™ cotton, do not present a plant pest risk and are therefore no longer regulated articles under its regulations at 7 CFR Part 340.

APHIS' determination has been made in response to a petition from Calgene, Inc., of Davis, California, received on July 15, 1993. The petition seeks a determination from APHIS that BXN™ cotton does not present a plant pest risk and is therefore not a regulated article. On September 8, 1993, APHIS announced receipt of the Calgene petition in the Federal Register (58 FR 47249-47250) 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 BXN™ cotton, food products derived from it, and the herbicide bromoxynil that may be used, if a new label for the herbicide is approved, in conjunction with it. APHIS invited written comments on whether BXN™ cotton poses a plant pest risk, to be submitted on or before November 8, 1993.

BXN™ cotton, as defined by its developer (Calgene, Inc., of Davis, California), is any cotton cultivar or progeny of a cotton line containing the BXN gene (a gene, derived from the soil microbe *Klebsiella pneumoniae* subsp. *ozaenae* that encodes the enzyme nitrilase, which can degrade the herbicide bromoxynil) with its associated regulatory sequences, i.e., sequences that allow for expression of the gene's enzyme product. By definition, BXN™ cotton may also contain: the *kan*^{r} marker gene (encoding the enzyme aminoglycoside 3'-phosphotransferase II, which confers resistance to the antibiotic kanamycin) with its associated regulatory sequences; a DNA fragment containing the origin of replication of the pRi plasmid from *Agrobacterium rhizogenes*; T-DNA left and right border sequences from an *Agrobacterium tumefaciens* Ti plasmid; a segment of DNA from transposon Tn*5*; a portion of a synthetic polylinker sequence from *lacZ'*; and a segment of DNA containing the origin of replication of plasmid pBR322. Expression of the BXN™ gene and the kan^r gene is directed by copies of the promoter from the *35S* gene from cauliflower mosaic virus and terminated using sequences derived from the *tml* gene from the octopine-type Ti plasmid pTiA6 from *A. tumefaciens*. Each of these sequences will be discussed in detail in section IV of this determination.

APHIS regulations at 7 CFR Part 340, which 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, 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, thereby allowing for unregulated introduction of the article in question.

BXN™ cotton lines have previously been field tested under 15 APHIS permits. Permitted field tests took place at a total of 57 sites in 13 states. Field tests were also conducted in Argentina, Bolivia, and South Africa in accordance with national regulatory requirements. Additional demonstration trials using BXN™ cotton were also performed under notification during the growing season just ended. All field trials were performed essentially under conditions of reproductive confinement.

BXN™ cotton contains components from organisms that are known plant pathogens, i.e., the bacterium *Agrobacterium tumefaciens* and cauliflower mosaic virus. BXN™ cotton has therefore been a regulated article under APHIS jurisdiction and its field tests in 1989, 1990, 1991, 1992, and 1993 have been in accordance with APHIS regulations. APHIS' determination that BXN™ cotton does 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 BXN™ cotton and observable properties of the cotton lines themselves. From this review, we have determined that these BXN™ cotton lines: (1) exhibit no plant pathogenic properties; (2) are no more likely to become a weed than their nonengineered parental varieties; (3) are unlikely to increase the weediness potential for any other cultivated plant or native wild species with which the organism can interbreed; (4) will not cause damage to processed agricultural commodities; and (5) are unlikely 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 BXN™ cotton lines bred from these lines will not exhibit new plant pest properties, i.e., properties substantially different from any observed for the BXN™ cotton lines already field tested, or those observed for cotton in traditional breeding programs.

Calgene has provided general information and data from field testing of some of the cotton lines fitting their definition of BXN™ cotton and intended to be representative of all those lines. Our determination, therefore, applies to cotton lines that fit Calgene's definition of BXN™ cotton and that have been field tested **under permit.** 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. Normal agronomic practices involving these BXN™ cotton lines, e.g., cultivation, propagation, movement, and cross-breeding with other cotton lines, can now be conducted without APHIS permit. [Importation of BXN™ 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 BXN™ 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; 44 FR 50381-50384; and 44 FR 51272-51274). An environmental assessment (EA) was prepared and a finding of no significant impact (FONSI) was reached by APHIS for the determination that BXN™ 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.

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 BXN™ cotton, as well as a summary of comments provided to APHIS on its proposed action; and (2) analysis of the key factors relevant to APHIS' decision that BXN™ cotton does not present a plant pest risk.

II. Background

USDA Regulatory Framework

APHIS regulations, which 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, 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 § 340.2 of the regulations and is also a 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 § 340.1 of the regulations to either (1) notify APHIS in accordance with § 340.3 or (2) obtain a permit in accordance with § 340.4. Introduction under notification (§ 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 § 340.4, a permit is granted for a field trial when APHIS has

determined that the conduct of the field trial, under the conditions specified by the applicant or stipulated by APHIS, does not pose 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 to demonstrate that although 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 for unregulated introduction of the article in question. A petition may be granted in whole or in part.

BXN™ cotton lines have been considered "regulated articles" for field testing under Part 340.0 of the regulations in part because of the vector system used to transfer the bacterial nitrilase gene into the recipient cotton. The vector system 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.

APHIS believes it prudent to provide assurance prior to commercialization that organisms, such as the BXN™ 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 BXN™ cotton is no longer a regulated article is based in part on evidence provided by Calgene concerning the biological properties of the BXN™ cotton and its similarity to other varieties of cotton grown using standard agricultural practices for commercial sale or private use. The BXN™ cotton has been field tested under 15 APHIS permits (92-106-01, 92-105-01, 91-357-01, 91-333-02, 91-329-04, 91-329-03, 91-329-02, 91-329-01, 91-107-06, 91-035-07, 90-303-02, 90-297-01, 90-016-04, 89-192-01, and 89-047-07). Permitted field tests took place at a total of 57 sites in the following 13 states: Alabama, Arizona, Arkansas, California, Georgia, Hawai'i, Louisiana, Mississippi, Missouri, North Carolina, South Carolina, Tennessee, and Texas.

Field tests were also conducted in Argentina, Bolivia, and South Africa in accordance with national regulatory requirements. Calgene, in appendix 4 of its petition request, has provided field data reports from all of the above listed field trials. Additional demonstration trials using BXN™ cotton were also performed under notification during the 1993 growing season.

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 pest or that plants or genes are pathogenic. The regulations instead have the premise that when plants are developed using biological vectors from pathogenic sources, use material from pathogenic sources, or pathogens are used as vector agents, that they should be evaluated to assure that there is not a plant pest risk (McCammon and Medley, 1990). For each APHIS performs a review that allows a verification of the biology and procedures used; assesses the degree of uncertainty and familiarity; and allows the identification of any hazards, should they be present and predictable. The overall aims of APHIS regulations in the Code of Federal Regulations at 7 CFR Part 340 are to allow for the safe testing of genetically engineered organisms under an appropriate level of oversight, and to enable any issues of potential or hypothetical risks to be addressed early enough in the development of the new organisms to allow for the safe utilization of the technology in agriculture.

A certification 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 which 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 unlikely 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 unlikely to harm other organisms, such as bees, that are beneficial to agriculture. Evidence has been presented by Calgene that bears on all of these topics. In addition, because the Calgene petition seeks a determination regarding

new cotton varieties containing the BXN™ gene, it should be established that there is a reasonable certainty that any new cotton varieties bred from BXN™ 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 BXN™ cotton lines already field tested.

Oversight by Other Federal Agencies

Environmental Protection Agency (EPA).

The EPA regulates the use of pesticide chemicals, including herbicides, 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." For a pesticide that is already registered, the use of the pesticide on a new crop plant (i.e., use on a crop for which the pesticide is not already registered) requires EPA approval of an amendment to the registration. 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. 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.

Although Buctril (bromoxynil) is currently registered as an herbicide for use on a number of crop plants, including small grains (wheat, barley, oats, rye, triticale), seedling alfalfa, corn, sorghum, flax, garlic, onions, mint, and grasses grown for seed and sod production, it is not currently registered for use on cotton. Any use of Buctril on cotton would require the approval by EPA of an amendment to the registration under FIFRA and a tolerance review under FFDCA. (There are established tolerances for bromoxynil on alfalfa, barley, cattle, corn, flaxseed, garlic, goats, grass, hogs, horses, mint hay, oats, onions, rye, sheep, sorghum, and wheat.) The Calgene petition states that Rhone-Poulenc Ag Company, the manufacturer of the herbicide Buctril, has submitted to the EPA an amended label and tolerance for Buctril use on transgenic cotton.

Food and Drug Administration (FDA).

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 BXN™ cotton is under the jurisdiction of the FDA.

A food additive petition (originally submitted as a request for advisory opinion, Docket 90A-0416, November 26, 1990), prepared by Calgene with regard to use of the kan^r gene in food, is pending at FDA.

III. Responses to Comments

APHIS received a total of 45 comments from State officials, universities, farmers associations and cooperative extension services, environmental and consumer organizations, and business and professional associations. Among these commenters, 34 were in favor of granting the petition, 9 were opposed, and 2 others addressed APHIS' decision on the petition itself only parenthetically.

At least two-thirds of the 34 comments in favor of granting the petition expressed the following views: that BXN™ cotton behaves no differently than traditional cotton varieties; that it is no more persistent or invasive than traditional varieties; that it offers the potential for decreased overall herbicide usage in cotton agriculture; and that it is a useful tool in integrated weed management systems. Each of the following assertions was made by two commenters: that the BXN™ cotton poses no plant pest risk; that it exhibits equal or increased yield and lint quality with decreased herbicide use; and that there is no chance that the BXN™ cotton varieties will be weedy. Additionally, one commenter noted that in consideration of the consequences of outcrossing of the transgenic cotton lines, it must be realized that such an eventuality is inevitable, but that management practices favor the maintenance of varietal purity, and in any event there is abundant nontransgenic cotton pollen present in cotton production areas. Another commenter indicated the importance of affirming the petition in enabling easier on-farm testing for more thorough evaluation of weed management systems and extension service demonstration projects. Another pointed out that the new cotton varieties could be important for conservation tillage in accordance with the Food and Security Act. One other comment indicated that the cauliflower mosaic virus regulatory sequences used to direct expression of the nitrilase and kanamycin resistance genes are harmless in these plants. APHIS is aware of no compelling contradictory data on any of these points, and concurs with these comments.

Of the nine comments opposing granting this petition, four indicated that the petition should be rejected because there is no strong Federal program, to which all transgenic crops should be subjected, to assess and minimize their risks. APHIS disagrees. The Coordinated Framework for Regulation of Biotechnology (51 FR 23303-23350; June 26, 1986), developed by the Office of Science and Technology Policy (OSTP), establishes a clear system for product-based coordinated reviews of the products of agricultural biotechnology. Roles are set out for the USDA, APHIS, EPA, and FDA, based on the existing legal authorities of the respective agencies for oversight over particular aspects

of this economic sector. These agencies are committed to working cooperatively to ensure adequate oversight for the products of agricultural biotechnology. The system is entirely adequate to identify and address any significant potential risks that may be posed by any of the new products of agricultural biotechnology. One comment suggested that APHIS' authority to regulate these products under the FPPA is questionable. APHIS also disagrees with this comment. Our responsibilities under the Act to protect against the introduction or dissemination of plant pests provide broad authority over any products that may have potentially significant impacts on the environment, based on the broad definition of "plant pest" in the statute.

Two comments indicated that transgenic BXN™ cotton poses a threat to ecological diversity akin to threats posed by the introduction of exotic plants into new environments. Three other comments asserted that in coming to a determination that BXN™ cotton has no potential to pose a plant pest risk, APHIS needs to consider potential effects of introduction of the transgenic cotton on centers of cotton diversity in developing nations. APHIS believes that it can be clearly established that BXN™ cotton poses no threat to ecological diversity in the United States. As will be discussed below, there is no reason to think that the diversity or prevalence of wild cotton relatives will be affected by introduction of BXN™ cotton, and wild cotton (*Gossypium hirsutum*), which would also likely be unaffected in any case, only grows at sites distant from areas of cotton production. The general matter of the putative analogy between the introduction of an exotic species into a new environment and the introduction of a genetically modified crop plant is not relevant to this determination because the attributes of BXN™ cotton fall within the ranges established for those traits in traditional cotton lines, except for tolerance to bromoxynil. Any impacts of that trait have been carefully considered.

APHIS has considered the concern about the potential effects of the cultivation of BXN™ cotton outside the United States. A general consideration of this topic indicates to us that there are no necessary impacts on cotton diversity occasioned by this determination to allow the cultivation, without permit, of BXN™ cotton in the United States. Even if BXN™ cotton were to be cultivated in agricultural regions around centers of cotton diversity, it seems extremely unlikely that BXN™ cotton would have any effect on wild progenitors of cultivated cotton. The herbicide bromoxynil is generally used only in agricultural contexts rather than on uncultivated land, and there is no reason to believe that the bromoxynil resistance trait would impart any selective advantage to a recipient plant in the absence of bromoxynil application. In any event, 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). Therefore, this topic will not be discussed in any greater detail. We would, however, note these additional facts: (1) crop plants and seeds exported from the United States, whether transgenic or nontransgenic 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 and/or commerce with transgenic cotton are unaffected by our action; and (4) APHIS has no jurisdiction over approval for the use of bromoxynil on cotton plants in foreign nations. Scenarios in which an impact of BXN™ 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.

One comment argued that if seeds of BXN™ cotton are to be exported, the United States government "should require that they be labeled to state that approval of the crop under U.S. law carries no implication of safe use in other countries." APHIS does not believe that such labelling is desirable. The commenter is correct, however, in indicating that this determination does not carry with it any foreign safety presumption, inasmuch as our authority only extends to the borders of the United States and its territories and possessions. We do not believe that the absence of such a labeling requirement will lead to any lack of clarity on this matter. With respect to the normal transit of agricultural commodities, USDA regulations in place require that certifications for export of those commodities meet the phytosanitary requirements of the importing nation. We as a signatory nation under the International Plant Protection Convention certify that movement of plants or plant materials out of the United States will not present the risk of injurious plant pests. The Agreement on Sanitary and Phytosanitary Measures in the General Agreement on Tariffs and Trade (GATT) also requires that the parties employ science based risk assessment methods for commodities. More specifically, it should be noted that APHIS is in frequent contact with agricultural officials from many foreign nations, including those with interest in the cultivation of genetically engineered cottons. We are actively involved with many countries as they develop national scientific and regulatory frameworks that will enable them to make their own scientifically credible decisions about the safety of new crop varieties.

Several comments indicated that Calgene did not adequately address the toxic or deleterious effects of the herbicide bromoxynil and the potential for its increased use on BXN™ cotton. Some comments also questioned Calgene's assertion that commercialization of BXN™ cotton would lead to decreased herbicide use. Since this determination does not authorize the use of bromoxynil on cotton, APHIS does not believe that this information is relevant to its decision. EPA has authority over the use in the environment of all pesticidal substances, including herbicides, under FIFRA; in particular, EPA has jurisdiction over registration of bromoxynil for use on cotton. Approval by EPA of a particular label condition for a pesticide is granted when, under the specified conditions of use, it will not generally cause unreasonable adverse effects on the environment. EPA considers both human health and safety as well as nontarget effects of both the herbicide and its breakdown products in making a decision on registration of an herbicide. (If EPA has reason to believe that metabolism in a new crop would be significantly different, additional studies may be required. Residues of the metabolites and their effect on humans would be evaluated whether the source of the metabolites

was from the plant or from the soil.) Questions regarding potential increased use of the herbicide bromoxynil and aggregate herbicide use on cotton are more properly addressed to that agency. Based on our knowledge of current cultivation practices and herbicide use on cotton, we expect that overall herbicide use on cotton would change modestly, if at all, if even a large proportion of cotton cultivated in the United States were to be tolerant to bromoxynil. A reduction in frequency of herbicide use might be seen in cases when bromoxynil can be applied postemergence and replace preplant treatments with other herbicides at the site. In any event, copies of all comments relating to potential adverse consequences arising from approval of a label for bromoxynil used on BXN™ cotton are being forwarded to EPA. Additionally, EPA intends to request public comment on the use of bromoxynil on cotton prior to its decision on granting a registration for this use.

One comment asserted that, in response to this petition, an environmental impact statement needs to be prepared in which APHIS must consider "every relevant environmental effect" of the commercialization of BXN™ cotton. The comment argued that there will be a significant impact to the environment based on increased use of bromoxynil with concomitantly increased potential for human health effects and environmental exposure of fish to the persistent aqueous breakdown product bromoxynil octanoate. This comment also noted that the burden is on APHIS to demonstrate that any impacts are insignificant. APHIS agrees that it has the responsibility to analyze the potential impacts of its determination, but notes that the basis for the determination is a question of fact, specifically, the plant pest status of an organism. We disagree that speculative impacts relating to the commercialization of BXN™ cotton are relevant. This determination does not authorize the use of bromoxynil on cotton, nor does it constitute a license to commercialize BXN™ cotton. Moreover, lint from BXN™ cotton plants grown under permit may currently be sold as fiber, because devitalized plant material is not considered a regulated article under our regulations. With respect to consideration of the impacts of increased bromoxynil use on the environment, we reiterate that EPA has the responsibility for ensuring that any uses of herbicide will not cause unreasonable adverse effects on the environment within the context of FIFRA. The potential issuance by EPA of a new label for use of the herbicide bromoxynil on BXN™ cotton and this determination by APHIS regarding the cotton itself are separate decisions, under consideration by different agencies based on distinct regulations under unrelated legal authorities in response to requests from two separate corporate entities. One comment asserted that approval of the petition would violate the White House commitment to reduce use of pesticides. APHIS disagrees. Again, we note that this determination is not an authorization for the use of bromoxynil on BXN™ cotton.

One comment expressed the opinion that the asserted benefits from the use of BXN™ cotton are speculative and disingenuous, while the real benefits are likely to be minimal or nonexistent. APHIS believes that any analyses of future benefits from the use of

BXN™ cotton would be speculative, but in any case are again irrelevant to the questions of fact addressed in this determination.

One comment asserted that APHIS has failed to establish "data requirements sufficient to permit it to conduct a scientifically credible risks [sic] assessment." APHIS disagrees. The data requirements spelled out in 7 CFR §340.6 (b) of our final rule of March 31, 1993, were established through notice and comment rulemaking with input from the public. Those data requirements put applicants on notice that they are required to address the widest variety of topics relating to effects both within and outside the agricultural milieu if there is any reason to consider that the subject organism would pose a greater plant pest risk than the unmodified organism from which it was derived. We do not believe that additional protection of human health or the environment would result from imposing broad testing requirements for all petitions involving transgenic plant varieties. Rather, we believe that it is more efficient and appropriate to require applicants to address whatever issues are raised based on the biology of the recipient organism and the donor genes. This may be accomplished through field tests, laboratory experimentation, agronomic observations, review of the scientific literature, consultations with experts, or other methods, depending on the nature of the concern.

Seven comments indicated that Calgene's data on the effects of gene flow to wild but nonweedy relatives such as *G. tomentosum* are inadequate or that the spread of the introduced genes to other plants could have serious (but unspecified) consequences or unknown ecological risks. APHIS disagrees that there is inadequate information on the issue of gene flow to weedy or nonweedy relatives to reach a decision on Calgene's petition. APHIS believes that there is no reason to require more testing than has been performed to date by Calgene. In order to come to a conclusion regarding the probability and consequences of gene transfer to wild relatives, APHIS has considered a great deal of available information. Some of the information considered relates to cotton biology, e.g., the documented lack of ability of cotton to overwinter in most of its growing areas (and the existence of regional requirements for cultivation practices to prevent its persistence in those areas, such as Arizona, where it can persist) and the known differences in the pollination behavior and habitats of *G. hirsutum* and the native Hawai'ian species, *G. tomentosum*, and of the most recent genetic and biochemical data relating to the lack of introgression of genes from *G. hirsutum* into the native species.

It is important to note that, while raising concerns about pollination of wild cottons by BXN™ cotton, none of the commenters identified a specific risk that would differentiate pollination by those cottons from pollination by other commercial varieties. Cotton lines bred by traditional means, which should be no more or less likely to interbreed with *G. tomentosum* than BXN™ 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. In addition, there is no reason to believe that a selective advantage will be conferred on hybrid progeny carrying the nitrilase or <u>kan</u>^{r} genes in the absence of bromoxynil or kanamycin

selection in the environment. The data provided by Calgene in its invasiveness studies are confirmatory of the predicted behavior of cotton. APHIS does not mean to imply by this discussion that it believes that gene flow to wild relatives cannot occur. Rather, gene flow to wild cottons could occur in some instances, particularly to wild *G. hirsutum*, but APHIS has concluded that these occasional events will not be of consequence. The introduced genes should provide no selective advantage to plants growing on noncultivated land that is not treated with bromoxynil.

Several of the comments also specifically noted that Calgene's invasiveness studies were conducted at only one site but should have been performed in a variety of environments. APHIS agrees that, as with any agronomic studies, data obtained at a variety of sites is useful in order to confirm that local environmental variations will not materially alter the performance of the test crop. The question of how much testing should be required to confirm negative results is a matter of balancing hypothetical risks against real financial burdens. While additional tests of the same type might be interesting, APHIS does not believe that additional studies at other sites are warranted in the absence of any indication that data obtained at other sites should be different.

One comment noted Calgene's description of wild populations of *G. hirsutum* as strand vegetation in tropical Americas rimming the Gulf of Mexico and extending into Florida "in virtual isolation from areas of agriculture," and indicated that this asserted isolation must be verified geographically. APHIS has investigated the distribution of wild *G. hirsutum*. According to Dr. Paul Fryxell of Texas A&M University (personal communication), who is 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. However, even if the nonagricultural land containing these wild cotton populations were near sites of commercial cotton production, APHIS believes that its 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, but have in fact been subject in the past to Federal eradication campaigns, because they can serve as potential hosts for the boll weevil, *Anthonomus grandis* Boh.

One comment contended that tests on seed germination have inappropriate controls, i.e., transgenic plants were compared with standard varieties rather than nontransgenic parental ones. APHIS believes this comment is in error. Because cotton varieties such as Coker are not genetically homogeneous, it is not possible to make exact comparisons with parent strains. There is noticeable variation among progeny produced even with propagation of a single Coker "strain" in a single cotton field. Therefore, it is most

appropriate to compare transgenic lines with the range of parentals to see whether the phenotype in question falls within the range expected for normal cotton varieties.

It was also suggested in this comment that: germination data obtained in greenhouses may not accurately reflect field germination of seeds; that the acid treatment used for delinting the seeds in some of the studies could possibly obscure some differences in dormancy; and that better studies would have involved the use of buried seeds. APHIS agrees that each of the first two assertions is possibly correct, and that buried seed studies might be preferable to the types of studies performed. However, APHIS does not believe that the cited imperfections in Calgene's studies are sufficient to cast into doubt the other data on BXN™ cotton. In particular, knowledge about the lack of persistence and weediness of cultivated cotton and about cultivation practices with the crop, and Calgene's field study on the lack of invasiveness of BXN™ cotton, indicate that additional dormancy, however unexpected, would not pose a plant pest risk.

Three comments noted that, while Calgene claimed that the properties of BXN™ cotton are unchanged from those of parental varieties except for the intended modifications, the company indicated in its discussions of its yield trials on page 135 of the petition that the transgenic strains may have smaller seeds. In these comments, the opinions were expressed that the observation itself merits examination and that the initial claim that BXN™ cotton does not differ from other varieties except for bromoxynil resistance is unsubstantiated. In addition, one of the comments noted that Calgene's data also indicated differences between some BXN™ lines and standard controls in weight of linters and hulls; and that in some of the BXN™ lines the levels of gossypol are not within the reported range for the toxin. In response, APHIS notes first that a considerable amount of variation is routinely observed during crop breeding. This is a normal and expected occurrence, particularly for crops such as cotton, in which the breeding stocks are not homogeneous, in the breeding process, varieties having desirable characteristics are selected for further development, while those exhibiting less desirable ones are excluded from it. Slight and unremarkable changes in agronomic properties such as may have been observed in some transformants are not unexpected and raise no grounds for concern. These changes are not, in APHIS' view, any indication that there might be as yet undiscovered pleiotropic effects of the introduced genes in BXN™ cotton plants. APHIS believes that the fact that BXN™ cotton lines may have very slightly smaller seeds, as inferred by Calgene, poses no plant pest risk. Calgene has, APHIS believes, correctly argued that while a relationship between seed size and increase in weediness potential may apply in small-seeded crops, in which seed dispersal is affected by factors like wind, it does not in large-seeded crops like cotton.

One comment asserted that, for completeness, the applicant should perform floristic studies and provide experimental data "that gene transfer to wild relatives will not occur under the unrestricted conditions of commercial use." APHIS disagrees. APHIS believes it is, in fact, possible that gene transfer could occur to wild relatives; it is likely that

gene transfer to feral *G. hirsutum* plants will occur. However, APHIS does not believe that such gene transfer events will cause any plant pest risk or significant impact to the human environment. There should be no appreciable selection for maintenance of the herbicide tolerance trait outside the agricultural environment on which bromoxynil is applied. Standard field management practices can control any herbicide tolerant cotton plants on agricultural land.

Of the remaining two comments, one noted that the commenter's State retains the right to regulate BXN cotton regardless of actions by APHIS but added that no adverse effects had been noted during 3 years of field testing of BXN cotton at multiple sites in his State. APHIS concurs, noting, for example, that the State has routine jurisdiction over intrastate quarantine matters and seed certification. The other comment requested that APHIS amend the Federal Plant Pest Act to include any organisms that can alter the environment, in order that there be no loopholes in oversight over scientific research. APHIS disagrees that there are any material deficits in its oversight authority. The definition of plant pest in our regulations is very broad and our regulations already allow that we may regulate genetically engineered plants that we have reason to believe cause plant pest risk.

IV. Analysis of the Properties of BXN™ 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 BXN™ cotton.

Biology and Cultivation of Cotton

Four species of the genus *Gossypium* are known as cotton, which 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 Hawai'i, 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 Hawai'i. *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). *Gossypium 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). Concentration of suitable pollinators varies from location to location and by season, and is considerably suppressed by insecticide use. If suitable bee pollinators are present, 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, depending 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 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 harboring of the insect. Weed management is a major concern in the cultivation of cotton. Weed management practices in cotton cultivation have changed over the years (Frans and Chandler, 1989; Ridgway et al., 1984). While hand hoeing was the primary means of weed control up through the 1950's, it has become a much more minor component with

the increasing cost of labor. Similarly, flame cultivation using butane or propane burners has also declined as fuel costs have risen (Ridgway et al., 1984). 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).

To reach its determination that BXN™ cotton does not present a plant pest risk, APHIS has analyzed not only public comments and basic information on the biology of cotton, but also data presented by Calgene and scientific data on other topics relevant to each of the considerations previously listed as relevant to a discussion of plant pest risk. Based on the data described, APHIS has arrived at a series of conclusions regarding the properties of BXN™ cotton.

(1) Neither the introduced genes, their products, nor the added regulatory sequences controlling their expression presents a plant pest risk in these BXN™ cotton plants.

The disarmed *Agrobacterium tumefaciens* transformation vector does not present a plant pest risk in BXN™ cotton. The vector system used to transfer the BXN™ gene into the cotton nuclear genome is based on the natural tumor-inducing (Ti) plasmid system used by the plant pathogenic bacterium *A. tumefaciens* for plant infection and gene transfer (Zambryski, 1988). (*A. tumefaciens* is the causal agent of a plant disease called crown gall.) Calgene has presented evidence that the Ti-plasmids that have been used in the construction of all BXN™ cotton lines that have been field tested (pBrx74 and pBrx75) have been disarmed, i.e., the natural pathogenicity genes which result in the characteristic symptoms of crown gall (e.g., overproduction of phytohormones in the plant resulting in unusual cell and organ overgrowth and the formation of galls, and synthesis of unusual, tumor-specific amino acids) in an infected plant have been removed from the transferred or T-DNA. The natural gene sequences between the T-DNA border sequences can be deleted and replaced by DNA from other sources without affecting the ability of *A. tumefaciens* to transfer the T-DNA to plants (Caplan

et al., 1983). Only the border sequences of the T-DNA are required for transfer into the plant nuclear genome and *generally* only DNA located between the border sequences is efficiently transferred and integrated (Wang et al., 1984); genes inserted into the T-DNA region by conventional cloning techniques will be transferred and integrated into the plant nuclear genome using this vector system (Hernalsteens et al., 1980). The vector system used by Calgene is said to be "binary," i.e., the genes to be transferred are found on one plasmid and the genes encoding functions necessary for transfer are found on a second plasmid.

The scientific literature, reviewed by Calgene for its field trials and previously evaluated by APHIS in environmental assessments relative to those field trials for BXN™ cotton under permit, indicates that only the T-DNA region is transferred into the plant genome and only the sequences contained between the border DNA sequences are integrated (Fraley et al., 1986). It has been established in the scientific literature that the border sequences do not remain intact during the process of insertion of T-DNA into the plant cell genome, and therefore the inserted DNA is no longer a functional T-DNA. In other words, the transferred T-DNA segment cannot be transferred a second time to a new recipient using the same mechanism that originally inserted it into the recipient plant genome (Zambryski et al., 1982). The plasmid vector by itself is not viable and can only replicate inside bacterial cells. Calgene has found, however, in examining the physical structures of integrated DNA in cotton transformants carrying the BXN™ gene, that in some transformation events the integration event has not utilized the DNA sequences of the right T-DNA border and additional sequences beyond the right T-DNA border may be integrated as well. The only additional sequences derived from outside the T-DNA borders that may be present in BXN™ cotton according to the definition include: an origin of replication fragment from T-DNA from the related bacterium *Agrobacterium rhizogenes*; an origin of replication derived from the bacterial plasmid pBR322; a DNA segment derived from transposon Tn5 from the bacterium *Escherichia coli*; and a synthetic polylinker sequence modified from the gene for B-galactosidase (lacZ'), also from *E. coli*. These segments will be considered in detail later in this section.

Calgene has presented evidence in table 4 of their petition that the transferred genetic material in BXN™ cotton is genetically stable and segregates in a Mendelian fashion, i.e., in a fashion consistent with integration of the added genetic material into nuclear chromosomal DNA. Calgene has also analyzed the physical structure of integrated BXN™ genetic material in several transformant lines (See figure 1, petition; and appendix 14). In addition to these direct analyses, there is a wealth of data in the scientific literature, some of which is presented by Calgene, showing that *A. tumefaciens* T-DNA with or without genes for tumorigenicity becomes integrated into nuclear chromosomal DNA as part of the gene transfer process. A single unconfirmed report has shown that T-DNA can insert into chloroplast DNA (de Block et al., 1985). As integrated pieces of plant chromosomes, T-DNAs are subject to the same rules governing chromosomal rearrangements and gene stability as other plant genes. Once integrated

into plant chromosomes (as no other type of T-DNA maintenance in transformed cell lines has been demonstrated), T-DNA becomes no different than naturally occurring plant genes in terms of stability, or potential ability to persist in the environment outside of direct progeny of transformed plants. The T-DNA containing the BXN™ gene is transmitted through mitosis and meiosis as a new Mendelian locus that is an integral part of the transformed plant's genome.

Following the use of the disarmed *Agrobacterium* vector system for cotton transformation, the bacterium has been killed with the antibiotic carbenicillin so that subsequent infection or transformation by it will not be possible (Fillatti et al., 1987). Calgene has further indicated in its field reports that none of the transgenic cotton plants show disease symptoms indicative of infection by *A. tumefaciens*.

The introduced coding regions do not confer a plant pest risk. The cotton plants have been transformed with the BXN™ gene, a gene encoding the enzyme nitrilase 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. There is no reason to believe that this gene or its protein product could impart any capability to a BXN™ cotton plant to cause disease or damage to any other plant. The BXN™ cotton plants have also been transformed with a kanamycin resistance (kan^r) gene. The kan^r gene encodes the enzyme aminoglycoside 3'-phosphotransferase II, which confers resistance to the antibiotic kanamycin. (The kan^r gene is also frequently referred to in the literature as neomycin phosphotransferase.) This gene was introduced as a marker, i.e., as a tag enabling identification of cotton cells that had concomitantly taken up the BXN™ gene. The kan^r gene was isolated from a transposon contained in a strain of *Escherichia coli* K12 (Beck et al., 1982; Jorgensen et al., 1979). *E. coli*, a common enteric bacterium found in the human gut, is not a regulated article. The kan^r gene has no involvement in plant disease or damage. Also, its use does not result in the presence of the antibiotic kanamycin in BXN™ cotton and does not imply that kanamycin will be used in the cultivation of cotton.

It has been reported (Kobayashi et al., 1993; Mahadevan, 1963) that a nitrilase enzyme is involved in the synthesis of the important plant auxin indole-3-acetic acid (IAA) in cruciferous plants. (The predominant pathway for IAA synthesis in plants does not involve a nitrile intermediate (Schneider and Wightman, 1978; Cohen and Bialek 1984).) A nitrilase enzyme is also found in plants in the mustard and banana families (Thimann and Mahadevan, 1964; Mahadevan and Thimann, 1964) and a gene encoding nitrilase has been cloned from the crucifer *Arabidopsis thaliana* (Bartling et al., 1992). There is no published evidence about the existence of a comparable nitrilase-mediated pathway for IAA synthesis among plants of the mallow family (such as cotton). Even if such a pathway exists in cotton, it is quite likely that introduction of the nitrilase gene from *K.*

Virulence genes on the *A. rhizogenes* pRi plasmid are responsible for the hairy root phenotype of bacterial infection. None of the virulence genes from pRi is contained on the 7.5 kb DNA segment that may be present in some BXN™ cotton lines. The genetic material derived from *A. rhizogenes* that may be present in some lines was introduced into the *A. tumefaciens* plasmid in order to allow the donor plasmids to be stably replicated in the latter bacterium. This segment of DNA encodes several polypeptides (from open reading frames called repA, repB, and repC) that are highly homologous to known proteins, involved in replication and stability of large plasmids in bacteria, that are found on an *A. tumefaciens* plasmid and other plasmids from enteric bacteria (Nishiguchi et al., 1987; Tabata et al., 1989; Mori et al., 1986; Theophilus and Thomas, 1987). Calgene has provided evidence that these genes are physically separate from the regions of pRi DNA known to be involved in plant disease (Jouanin et al., 1985). In addition, there are no plant-specific control sequences present to allow expression of these genes. Therefore, these genes have no potential to cause any disease symptoms in the recipient cotton plants.

DNA sequences from the plasmid pBR322 (Sutcliffe, 1979) may also be present in some BXN™ cotton lines. The pBR322 origin of replication sequence is present to facilitate replication of the vector agent plasmid in *E. coli*.

Despite the presence of certain pathogen-derived sequences in the BXN™ genome, no crown gall, hairy root, or CaMV disease symptoms were observed by Calgene in any BXN™ cotton plants during greenhouse or field studies. Calgene further provides evidence that expression of any of the introduced genes does not result in disease symptoms or the synthesis of products toxic to other organisms. Levels of toxins normally found in cotton, such as gossypol and cyclopropenoids, appear to fall within normal levels. None of the regulatory sequences encodes any polypeptide product.

Calgene has also monitored its BXN™ cotton field trials to verify that the disease susceptibility of its transgenic plants did not differ from that of parental varieties. No difference in disease susceptibility was observed for the following diseases: damping-off diseases caused by *Phytophthora*, *Pythium*, and *Rhizoctonia* species; *Fusarium* and *Verticillium* wilts; and bacterial blight caused by *Xanthomonas campestris*.

There is no published evidence for the existence of any mechanism, other than sexual crossing of compatible *Gossypium* species, by which these genetic sequences can be transferred to other organisms. Comparative analyses of numerous gene sequences from microorganisms and plants to our knowledge have never yielded any published evidence of strong inter-kingdom gene homologies that would be indicative of recent or frequent gene exchanges between plants and microorganisms, except for *Agrobacterium*-mediated gene transfers. A certain amount of information can be found in the scientific literature (e.g., Carlson and Chelm, 1986; Wakabayashi et al., 1986; Doolittle et al., 1990) that provides a suggestion that transfer of genes from plants to microorganisms may have occurred over evolutionary time, i.e., in the eons since the various times of

divergence between the kingdoms. A single report (Bryngelsson et al., 1988) has suggested that plant DNA can be taken up by a parasitic fungus, but no further evidence has ever been forthcoming that such DNA uptake has resulted in the transfer of a functional DNA sequence. Additionally, it has been recently observed (Stierle et al., 1993) that both the Pacific yew (*Taxus brevifolia*) and an endophytic fungus (*Taxomyces andreanae*) found in its inner bark both produce the unusual anti-cancer substance taxol, but there is no published information available about the homologies between the taxol-synthesizing enzymes from the two organisms.

Even if a rare plant-to-microbe gene transfer were to take place, there is no reason to believe that such a transfer of any of the sequences, including the kan^r gene or BXN™ gene, would pose any plant pest risk. Also, in its petition to APHIS, Calgene has presented a calculation of the potential contribution of kanamycin-resistant bacteria derived by horizontal gene movement from the genome of the genetically engineered cotton based on a worst case scenario which starts with the premise that gene transfer will undoubtedly occur. Based on these calculations, they conclude that kanamycin resistant soil bacteria arising from transformation from plant debris would represent no more than 1.4×10^{-11}% of the kanamycin resistant microbes already present. Based on Calgene's calculations, as well as data in the scientific literature, we conclude that concerns regarding DNA transfer from BXN™ cotton to microorganisms are at best entirely speculative.

(2) BXN™ 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 (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 allotetraploid 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 trait of interest, bromoxynil tolerance, is unlikely to increase weediness of this cotton. Bromoxynil would not be applied on BXN™ cotton for the purpose of controlling the cotton itself, but rather for controlling unrelated weeds in the field. To increase weediness of the cotton plant there would have to be selection pressure on BXN™ cotton (Tiedje et al., 1989; Office of Technology Assessment, 1988) associated with bromoxynil use on it. Because bromoxynil will not affect the survival of BXN™ cotton and because *G. hirsutum* is not itself weedy, this type of selection pressure does not now and is unlikely ever to exist. Even if bromoxynil-resistant weedy plants were ever observed, bromoxynil treatment would not be the control method of choice; many other methods of control would be readily available.

Examination of the new genetic sequences besides the gene encoding bromoxynil 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.

Calgene's data from greenhouse studies show variability in germination rates among transgenic seed lines but no evidence of specific changes in the rate from parent to transgenic plant. Calgene's burial study shows no obvious increase in volunteer plants from buried seeds. In addition, Calgene's field reports show no obvious increase in volunteers from seed, regrowth from stubble, or increase in seed dormancy. Calgene does report on lint characteristics which may suggest a decrease in seed size. If such a decrease were real, APHIS believes that no competitive advantage affecting weediness would be conferred on the transgenic plants by this change. Calgene has correctly argued, 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.

(3) BXN™ cotton will not increase the weediness potential of any other plant with which it can interbreed.

As discussed under the section on lack of weediness of *G. hirsutum*, neither *G. barbadense*, *G. thurberi*, nor *G. tomentosum* shows 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 only include *G. hirsutum*, *G. barbadense*, and *G. tomentosum*.

BXN™ 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 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. Even if the nonagricultural land containing these wild cotton populations were near sites of commercial cotton production, this determination would not be altered, APHIS believes, because: (1) any potential effects of the trait would not alter the weediness of the wild cotton; (2) no authorization exists, nor is any sought, for the use of bromoxynil on nonagricultural land; and (3) the wild cotton populations in Florida are not being actively protected, but have in fact been 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 native diploid from Arizona with a DD genome, is not compatible with *G. hirsutum* pollen, so that BXN™ 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 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. There was a report (Fryxell, 1979) 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, 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 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 BXN™ 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 BXN™ 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 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. 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 BXN™ cotton to cultivated or occasional noncultivated *G. barbadense* would therefore not likely occur at a high level. Any movement of genetic material from BXN™ 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 in the absence of sustained bromoxynil use.

Should a movement of genetic material take place to these receptive plants and bromoxynil resistance be transferred, no competitive advantage would be conferred, because bromoxynil is not used with these plants when they are found in nonagricultural areas. In agricultural areas, such plants would be controlled by normal agronomic practices.

(4) BXN™ cotton will not cause damage to processed agricultural commodities.

Information provided by Calgene regarding the components and processing characteristics of BXN™ cotton revealed no differences in any component that could have an indirect plant pest effect on any processed plant commodity. Calgene evaluated the effects of the genetic modifications on BXN™ cotton by measuring fiber characteristics, seed processing characteristics, and the biochemical composition of oil and meal. Fiber characteristics were measured and the results were reported in the literature (Baldwin et al., 1992; Kiser and Mitchell, 1991). Fiber characteristics were measured and compared with Coker 315 control plants for nine lines of BXN™ cotton in 1991 and two lines in 1992. Fiber characteristics measured included micronaire (a measure of fiber fineness), length, uniformity ratio, strength, elongation, leaf index, and color factors. These measured fiber characteristics varied between the lines but were within the range of the controls. Cottonseed is processed into four major products: oil, meal, hulls and linters (Cherry and Leffler, 1984). For the evaluation of seed processing characteristics, Calgene presented data on the processing of delinted seeds from three lines of BXN™ oil, meal, hulls and linters. Although these data show considerable variability among the lines tested, the results were comparable to those from control plants. Calgene presented data on the composition of oil and meal derived from BXN™ cotton seed. Oil derived from nine BXN™ cotton lines was compared to oil derived from three Coker 315 controls and to a refined food grade cotton seed oil. The composition of the oil derived from the nine BXN™ cotton lines was comparable to the Coker 315 oil and the refined food grade oil. The measured values for all of the oils were within the expected ranges for standard, edible cottonseed oil, according to Codex Stan 22-1981. Total protein, total nitrogen and residual oil content of cottonseed meal were compared for three BXN™ cotton lines and two Coker 315 controls. These measured components varied between the lines but were comparable to controls.

(5) BXN™ cotton will not be harmful to beneficial organisms, including bees.

There is no reason to believe that deleterious effects on beneficial organisms could result specifically from the cultivation of BXN™ cotton. The novel proteins that will be expressed in the BXN™ cotton, nitrilase and aminoglycoside 3'-phosphotransferase II, are not known to have any toxic properties. Calgene has provided data to show that the expression levels of the two proteins in cotton leaves are less than 0.002 percent for nitrilase and less than 0.008 percent for aminoglycoside 3'-phosphotransferase II. 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. Additionally, Calgene provides data to show that the introduced nitrilase has a high specificity for bromoxynil (3,5,-dibromo-4-hydroxybenzonitrile). The high specificity of this enzyme makes it unlikely that the nitrilase would metabolize endogenous substrates to produce compounds toxic to beneficial organisms. APHIS has not identified any other potential mechanisms for deleterious effects on beneficial organisms.

IV. Conclusion

APHIS has determined that cotton plants fitting the definition of BXN™ cotton that have previously been field tested under permit will no longer be considered regulated articles under APHIS regulations at 7 CFR Part 340. Permits under those regulations will no longer be required from APHIS for field testing, importation, or interstate movement of those cotton lines or their progeny. (Importation of BXN™ 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 unlikely 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 unlikely to harm other organisms, such as bees, that are beneficial to agriculture. APHIS has also concluded that there is a reasonable certainty that new progeny BXN™ cotton varieties

bred from these lines will not exhibit new plant pest properties, i.e., properties substantially different from any observed for the BXN™ cotton lines already field tested, or those observed for cotton in traditional breeding programs.

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