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Inspection
Service



**Determination of Plant
Pest Risk for Insect-
Resistant and Glufosinate
Ammonium-Tolerant
(TwinLink™) Cotton,
Gossypium hirsutum,
events T304-40 x GHB119**

Petition 08-340-01p

**Draft
Plant Pest Risk Assessment
April 2011**

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Plant Pest Risk Assessment of Bayer CropScience TwinLink™ Cotton

A. Introduction

Bayer CropScience (BCS) has petitioned APHIS for a determination that TwinLink™ cotton is unlikely to pose a plant pest risk and, therefore, is no longer a regulated article under regulations at 7 CFR part 340. Bayer CropScience (BCS) has developed cotton (*Gossypium hirsutum*) plants that express two insecticidal proteins, Cry1Ab and Cry2Ae, from a common soil bacterium, *Bacillus thuringiensis* (Bt) and also expresses the *bar* gene that confers resistance to glufosinate ammonium herbicide from *Streptomyces hygroscopicus*. The Cry1Ab and Cry2Ae protein are effective in controlling lepidopteran larvae such as bollworm (CBW, *Helicoverpa zea*), tobacco budworm (TBW, *Heliothis virescens*) larvae and fall armyworm (FAW, *Spodoptera frugiperda*) which are common pests of cotton. In addition to the Cry1Ab and Cry2Ae proteins, TwinLink™ cotton also contains the PAT (phosphinothricin-acetyl-transferase) enzyme, encoded by the *bar* gene. This is the same protein that is expressed in Bayer CropScience Liberty Link cotton® (LLCotton25) that confers to the plant tolerance to the herbicide glufosinate ammonium.

B. History of Development of TwinLink™ Cotton

In 2002, APHIS deregulated the first phosphinothricin (glufosinate)-tolerant cotton, event GHB119; Aventis CropScience cotton designated Liberty Link cotton® (LLcotton25). This event involved cotton that was transformed with a binary plasmid vector carry the *bar* gene construct within a disarmed transfer DNA (T-DNA) from *Agrobacterium tumefaciens*. Aventis CropScience (now Bayer CropScience) completed its food safety consultation with the U.S. Food and Drug Administration (FDA) on Liberty Link cotton® in 2003 (<http://www.accessdata.fda.gov>; BNF 000086). Tolerance exemptions have been granted for the plant-incorporated protectant in Liberty Link cotton® (event LLCotton25) and the genetic material necessary for its production. On April 11 1997, the Environmental Protection Agency (EPA) granted exemptions from the requirement of a tolerance for residues of the plant-pesticide phosphinothricin-acetyl-transferase protein in or on food and feed commodities of cotton (62 FR 17719; 40 CFR 180.473). The Australia and New Zealand Food Authority (ANZFA) also completed a Toxicological Review and Risk Assessment, and a Technical Report was published in 2005 (ANZFA Application A533, 2005; <http://www.foodstandards.gov.au>). ANZFA concluded that Liberty Link cotton® was comparable to non-genetically engineered (non-GE) cotton in terms of their safety and nutritional adequacy. In 2004, the Plant Biosafety Office (PBO) of the Canadian Food Inspection Agency (CFIA) concluded that Bayer CropScience's Liberty Link cotton® was substantially equivalent to currently grown cotton, in terms of their potential environmental impact and livestock feed safety and the novel traits would not have any substantial negative effect on the environment (CFIA Decision Document DD2004-49; <http://www.inspection.gc.ca>).

Bayer CropScience has now developed the transgenic cotton, TwinLink™, by crossing cotton event GHB119 that expresses both the Cry2Ae protein and phosphinothricin-acetyl-transferase

(found in LiberyLink® cotton) proteins now with a newly developed cotton line (event 304-40) that expresses both Cry1Ab protein and phosphinothricin-acetyl-transferase (found in LiberyLink® cotton) proteins. TwinLink™ cotton has been developed by BCS as an alternative insect resistant and herbicide tolerant cotton product.

TwinLink™ cotton has been field tested under APHIS regulations since 2005. Data were provided in the petition for field trials completed prior to the petition submission. Field test reports can be found in the BCS TwinLink™ cotton petition in Appendix 1, p.102.

On December 22 2008, BCS submitted a request that the Environmental Protection Agency (EPA) grant an exemption from the requirement of a tolerance for residues of the plant-incorporated protectant (PIP), *Bacillus thuringiensis* Cry2Ae and Cry1Ab insect control proteins and the genetic material necessary for its production, in or on all food commodities. BCS has also submitted a food safety summary to FDA indicating that food and feed derived from TwinLink™ cotton are as safe and nutritious as food and feed derived from conventional cotton.

1. Description of the modifications

TwinLink™ cotton contains the stably integrated genes *cry1Ab*, *cry2Ae* and *bar*, which encode respectively the Cry1Ab, Cry2Ae and PAT proteins. “The genes were introduced by *Agrobacterium*-mediated gene transfer. Southern blot analyses show TwinLink cotton contains one complete copy of the *cry1Ab* and *cry2Ae* genes, and 2 copies of the *bar* gene” (pg 2, BCS petition).

The two parent lines that were conventionally crossed to produce TwinLink™ cotton contain the following genetic components (also described in BCS petition on page 21):

Cry2Ae + PAT (event GHB119) has been genetically engineered to contain the following transgene fragments:

- Left border (LB)** repeat from the T-DNA of *A. tumefaciens* (Zambryski 1988).
- 3' nos:** sequence including the 3' untranslated region of the nopaline synthase gene from the T-DNA of pTiT37 in counter clockwise direction (Depicker, Stachel et al. 1982)
- bar;** the coding sequence of the phosphinothricin acetyltransferase gene of *Streptomyces hygroscopicus* in counter clockwise direction (De Block, Botterman et al. 1987)
- Pcsvmv XYZ** sequence including the promoter region of the Cassava Vein Mosaic Virus in counter clockwise direction (Verdaguer, De Kochko et al. 1996)
- P35S2** sequence including the promoter region from Cauliflower Mosaic Virus 35S transcript in clockwise direction (Odell, Nagy et al. 1985)
- 5'cab22L** sequence including the leader sequence of the chlorophyll a/b binding protein gene from *Petunia hybrida* in clockwise direction (Harpster, Townsend et al. 1988)
- TPssuAt** coding sequence of the transit peptide of the ribulose-1,5-biphosphate carboxylase small subunit gene *ats1A* of *Arabidopsis thaliana* in clockwise direction (De Almeida, Gosselé et al. 1989)
- Cry2Ae** the coding sequence of an insecticidal protein gene of *Bacillus thuringiensis*, adapted to cotton codon usage in clockwise direction

3'35S sequence including the 3' untranslated region of the 35S transcript of Cauliflower Mosaic Virus in clockwise direction (Sanfaçon, Brodman et al. 1991)
Right border (RB) repeat from the T-DNA of *A. tumefaciens* (Zambryski 1988)

Cry1Ab + PAT (event 304-40) has been genetically engineered to contain the following transgene fragments:

Right border (RB) repeat from the T-DNA of *A. tumefaciens* (Zambryski 1988)
3' me1 sequence that includes the 3' untranslated region of the NADP-malic enzyme gene of *Flaveria bidentis* (yellowtop) in counter clockwise direction (Marshall, Stubbs et al. 1996)
Cry1Ab sequence from *B. thuringiensis* berliner 1715 in counter clockwise direction (Höfte, de Greve et al. 1986)
5'e1 sequence that includes the leader sequence of the tapetum specific E1 gene (GE1) of *Oryza sativa* (rice) in counter clockwise direction (Michiels F. 1992)
Ps7s7 sequence including the duplicated promoter region derived from subterranean clover stunt virus genome segment 7 in counter clockwise direction (Boevink, Chu et al. 1995)
P35S3 sequence including the promoter region from Cauliflower Mosaic Virus 35S transcript in clockwise direction (Odell, Nagy et al. 1985)
bar; the coding sequence of the phosphinothricin acetyltransferase gene of *Streptomyces hygroscopicus* in clockwise direction (De Block, Botterman et al. 1987)
3' nos sequence including the 3' untranslated region of the nopaline synthase gene from the T-DNA of pTiT37 in clockwise direction (Depicker, Stachel et al. 1982)
Left border (LB) repeat from the T-DNA of *A. tumefaciens* (Zambryski 1988)

C. Plant Pest Risk Assessment

This plant pest risk assessment is to determine whether BCS TwinLink™ cotton is unlikely to pose a plant pest risk. If APHIS determines that a GE organism is unlikely to pose a plant pest risk, APHIS then has no regulatory authority over that organism under 7 CFR part 340.

APHIS administers the regulations 7 CFR part 340 under the authority of the plant pest provisions of the Plant Protection Act of 2000 (PPA).

The PPA states that:

“The term ‘plant pest’ means any living stage of any of the following that can directly or indirectly injure, cause damage to, or cause disease in any plant or plant product:

- (A) A protozoan.
- (B) A nonhuman animal.
- (C) A parasitic plant.
- (D) A bacterium.
- (E) A fungus.
- (F) A virus or viroid.

- (G) An infectious agent or other pathogen.
- (H) Any article similar to or allied with any of the articles specified in the preceding subparagraphs.”

The Animal and Plant Health Inspection Service of the United States Department of Agriculture (USDA-APHIS) has prepared a Plant Pest Risk Assessment in response to a petition (APHIS Number 08-340-01p) from BCS. APHIS regulation 7 CFR 340.6(c) stipulates the information needed for consideration in a petition for nonregulated status. APHIS evaluated information submitted by the applicant related 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, any impacts on the weediness of any other plant with which it can interbreed for BCS TwinLink™ cotton. Issues related to agricultural or cultivation practices and the effects of the regulated article on non-target organisms are considered in the Environmental Assessment for BCS TwinLink™ cotton.

Based on information on the biology of cotton (OECD 2008) data presented by BCS (APHIS Number 08-340-01p) and scientific data relevant to a discussion of plant pest risk, APHIS concluded the following regarding BCS TwinLink™ cotton:

1. Potential impacts of altered disease and pest susceptibilities

USDA-APHIS assessed whether BCS TwinLink™ cotton is likely to have significantly increased disease and pest susceptibility. The assessment encompasses a thorough consideration of introduced traits and interactions with pest and disease.

Cotton (*Gossypium hirsutum*) is not a plant pest in United States, and the introduced DNA in BCS TwinLink™ cotton is unlikely to pose a plant pest risk because there are no pathogenic DNA sequences present. The description of the genetic modifications, including genetic elements, expression of the gene product and their functions for BCS TwinLink™ cotton has been summarized above. Because the goal of TwinLink™ cotton was to decrease the pest susceptibility, the data submitted by BCS indicated significant differences between BCS TwinLink™ cotton and the non-transgenic counterparts for insect stresses (as measured by 32 agronomic parameters evaluated found on page 68 of the petition). An increase in yield was attributed to the inserted gene expression in TwinLink™ cotton. There were no increases in pest susceptibility or disease as measured by the agronomic properties found on page 68 of the petition.

The introduced genes (*cry1Ab*, *cry2Ae* and *bar*) encode for Cry1Ab and Cry2Ae and glufosinate synthase proteins, respectively. The *cry1Ab* and *cry2Ae* genes come from the soil bacteria *Bacillus thuringiensis* and the *bar* gene is from *Streptomyces hygroscopicus*. Such genes have been used in field trials previously and are not known to cause plant disease. There is no indication that inserting the *cry1Ab*, *cry2Ae* and *bar* genes will result in increased likelihood of introduction or dissemination of a plant pest. Cry proteins were isolated and used commercially as a pesticide in France in 1938, marketed under the name Sporine. Since 1961, EPA approved Bt proteins for the treatment against lepidopteran pests under the trade name, Thuricide (UCSD 1999; Erlandson and Litowski 2005). APHIS has not identified any plant pest risk following the introduction of Cry proteins or phosphinothricin acetyltransferase in prior deregulated products,

such as rice, corn, cotton, beet, rapeseed, or soybean. A list of petitions of nonregulated status granted can be found at http://www.aphis.usda.gov/brs/not_reg.html. The Plant Biosafety Office (PBO) of the Canadian Food Inspection Agency (CFIA) also concluded that the intended effects herbicide-resistant plants such as phosphinothricin acetyltransferase are not related to altered plant pest potential (<http://www.inspection.gc.ca/english/sci/biotech/enviro/herbice.shtml>). CFIA has also reviewed the plant pest risk, the environmental risk and safety of plants with novel genes containing Cry proteins and found these proteins are not related to altered plant pest potential. CFIA's decision documents can be found at <http://www.inspection.gc.ca/english/plaveg/bio/dde.shtml>.

2. Potential impacts from outcrossing of the BCS TwinLink™ cotton to wild relatives

In assessing the risk of gene introgression from BCS' TwinLink™ cotton into its sexually compatible relatives, APHIS considers two primary issues: 1) the potential for gene flow and introgression via pollen movement and horizontal gene transfer¹; and 2) the potential plant pest risk of introgression.

a) Gene Flow via Pollen Movement

Movement of genetic material by pollen is possible only to those plants with the proper chromosomal type. In the United States, this would only include *G. hirsutum*, *G. barbadense*, and *G. tomentosum*. Native *G. barbadense* is only found in Hawaii, Virgin Islands and Puerto Rico, while *G. tomentosum* is only found in Hawaii (Fryxell 1979). *G. hirsutum* is generally self-pollinating but some cross-pollination can occur, albeit at relatively low incidence through activity of pollinating insects (Fryxell 1979). Gene movement between *G. hirsutum* and *G. barbadense* is possible if suitable insect pollinators are present, and if there is a short distance from host plants to recipient plants (Fryxell 1979). Physical barriers, intermediate pollinator-attractive plants, and other temporal or biological impediments (geography or absence of pollinators) reduce the potential for pollen movement (Fryxell 1979). Table 1 outlines the compatibility of all species on an international level. Concentration of suitable pollinators varies from location to location and by season, and is considerably suppressed by insecticide use.

Table 1. Cotton Species

Species	Common Name	Native location	Comments
<i>G. hirsutum</i>	Upland cotton	Central America, Mexico, Caribbean and southern Florida.	Commercial Species, Grown in U.S.A. and comprises 97% of U.S.A cotton crop. Sexually compatible with <i>G. barbadense</i> and <i>G. tomentosum</i> .
<i>G. barbadense</i>	Pima, Creole, Egyptian or Sea Island cotton	S. America	Commercial species, grown in U.S.A. Grown in Hawaii, Virgin Islands and Puerto Rico. Sexually compatible with <i>G. hirsutum</i> and <i>G. tomentosum</i> .

¹ Horizontal gene transfer is any process in which an organism transfers genetic material to another cell that is not its offspring.

<i>G. tomentosum</i>	Ma'o or Hawaiian cotton	Hawaii	Non-commercial species. Thought to be only pollinated by moths when the flowers open at night, but more recent evidence suggests it can also be pollinated in early morning by bees. Only found in Hawaii. Sexually compatible with <i>G. hirsutum</i> and <i>G. barbadense</i> .
<i>G. arboreum</i>	Asiatic tree or tree cotton	Pakistan, India	Commercial species, grown in Europe, Africa and eastern countries. Sexually compatible with <i>G. herbaceum</i> .
<i>G. herbaceum</i>	Levant cotton	Africa, Arabia	Commercial species, grown in Europe, Africa and eastern countries. Sexually compatible with <i>G. arboreum</i> .
<i>G. thurberi</i>	Thurber's, Desert or Arizona desert cotton	Mexico, Arizona	Non-commercial species. Sexually compatible with <i>G. arboreum</i> and <i>G. herbaceum</i> .

Historically, it was reported that cross-pollination between *G. tomentosum* and *G. hirsutum* was unlikely because they use different insect pollinators and are receptive to pollination at different times of the day (McGregor 1976). Field and laboratory studies demonstrated that the historic literature is incorrect, and that these three species share common pollinators, and further that differences in flower structure and flowering habits do not serve as barriers to cross pollination (Pleasant and Wendel 2010). However, DNA marker analyses have not found evidence of genes from *G. hirsutum* occurring in native populations of *G. tomentosum* (DeJode and Wendel 1992). It is possible that the lack of evidence of movement of *G. hirsutum* genes into *G. tomentosum* is the result of lack of opportunity because cotton has not been grown commercially in Hawaii for at least the last 45 years (USDA-APHIS 2010).

In farm scale studies using traditional Upland cotton in California, it was found that the out-crossing distance was strongly dependent on the presence of bee colonies. When only native pollinators were present in the field, 1% out-crossing was detectable over a distance of 1 meter (approximately 3 ft) and 9 m (29.5 ft) when there was high pollinator activity (Van Deynze, Sunderstrom et al. 2005). Out-crossing declined exponentially with increasing distance from the source plot (Van Deynze, Sunderstrom et al. 2005). Current cultivation practices to prevent out-crossing (distance being primarily used) have been deemed sufficient to prevent unwanted gene flow. For Upland cotton, the Association of Official Seed Certifying Agencies (AOSCA) mandates an isolation distance being a nature barrier or crop boundary with a minimal isolation distance of 100 ft “if the contaminating source differs by easily observed morphological characteristics from the field to be inspected”. For Pima or Egyptian type cotton “the isolation shall be 1320 feet from any other type of cotton for Foundation and Registered and 660 feet for Certified seed”². Since TwinLink™ cotton is not morphologically distinguishable from traditional Upland cotton much like Pima or Egyptian type cotton, cultivation practices using AOSCA standards of 1320 ft for Foundation and Registered and 660 ft for Certified seed are used.

² From AOSCA “Yellow Books” 2003 OPERATIONAL PROCEDURES, CROP STANDARDS AND SERVICE PROGRAMS PUBLICATION (Genetic and Crop Standards), pg 194.

Wind is rarely seen as a means for cross-pollination of cotton pollen because of its adherent properties and large size (mean diameter of 53-56 µm). The pollen of cultivated *Gossypium* species is described as being sticky and having pronounced spines, with a marked tendency for groups of pollen grains to clump together (Hutmacher and Wright 2006).

b) Gene Flow via Horizontal Gene Transfer³

Transfer and expression of DNA from TwinLink™ cotton to soil bacteria is unlikely to occur. Gebhard and Smalla (Gebhard and Smalla 1999) and Schlüter *et al.* (Schlüter, Fütterer *et al.* 1995) have studied transgenic DNA movement to bacteria, and although theoretically possible, determined mathematically it would occur at extremely low rates (approximately 1 in 10⁻¹⁴). Many genomes (or parts thereof) have been sequenced from bacteria that are closely associated with plants including *Agrobacterium* and *Rhizobium* (Kaneko, Nakamura *et al.* 2000) and there is no evidence for recent horizontal transfer. Koonin *et al.* (Koonin, Makarova *et al.* 2001) and Brown (Brown 2003) presented reviews based on sequencing data that revealed horizontal gene transfer occurs occasionally on an evolutionary time scale of millions of years. Even in the unlikely event transfer were to occur, the gene would be poorly expressed at best because transgene promoters and coding sequences are optimized for plant expression and function poorly in prokaryotic cells.

Based on the above considerations, TwinLink™ cotton will not adversely impact sexually compatible wild relatives or their weediness characters.

3. Potential impacts based on the relative weediness of BCS TwinLink™ cotton.

In the United States, cotton is not listed as a weed in the major weed references (Crockett 1977; Holm, Pancho *et al.* 1979; Muenscher 1980), nor is it present on the list of noxious weed species (7 CFR 360) distributed by the Federal Government (USDA-APHIS 2006). Furthermore, cotton has been grown throughout the world without any report that it is a serious weed. Cotton is unlikely to become a weed. It is not persistent in undisturbed environments without human intervention. In the year following cultivation, cotton may grow as a volunteer only under specific conditions (disturbed or cultivated soil that had cotton grown in the last growing season) and can be easily controlled by herbicides or mechanical means. It does not compete effectively with cultivated plants or primary colonizers because it is such a slow grower, especially in the cooler soils in the northern cotton belt (OECD 2004).

APHIS assessed whether TwinLink™ cotton is any more likely to become a weed than the isogenic nontransgenic cotton line, or other cotton varieties currently under cultivation. The assessment encompasses a thorough consideration of the basic biology of cotton and an evaluation of the unique characteristics of TwinLink™ cotton evaluated under field conditions.

BCS conducted agronomic field trials during the 2005, 2007 and 2008 growing seasons across 8 states and 23 locations representative of the major cotton-growing areas of the cotton belt (field test reports can be found in the BCS TwinLink™ cotton petition in Appendix 1, p.102). The data

³ Horizontal gene transfer is any process in which an organism transfers genetic material to another cell that is not its offspring.

submitted by BCS indicated no significant differences between BCS TwinLink™ cotton and the non-transgenic counterparts (as measured by 32 agronomic parameters evaluated found on page 68 of the petition).

Based on the agronomic field data and literature survey about cotton weediness potential, TwinLink™ cotton lacks ability to persist as troublesome weed, and there would be no direct impact on current weed management practices for cotton cultivation.

4. Potential impacts on target and nontarget organisms, including beneficial organisms.

The Cry1Ab and Cry2Ae proteins expressed in TwinLink™ cotton are effective in controlling specific lepidopteran larvae such as bollworm (CBW, *Helicoverpa zea*), tobacco budworm (TBW, *Heliothis virescens*) larvae and fall armyworm (FAW, *Spodoptera frugiperda*) which are common pests of cotton. These Cry proteins are not expected to adversely affect non-target invertebrates such as bees, ladybugs, lacewings, spiders or other arthropods and vertebrate organisms, including birds, mammals and humans, because they do not contain the receptor found in the midgut of target insects (OECD 2007).

Cry1Ab and Cry2Ae are crystal delta-endotoxins from *Bacillus thuringiensis*. A total of 314 different *cry* genes have been identified by 2005 (OECD 2007). The insecticidal activity of Cry1Ab and Cry2Ae proteins occurs when the C-terminal half of these inactive protoxins are enzymatically cleaved within the midgut of susceptible insect larvae by trypsin-like proteases to the active toxin, which consists of the N-terminal portion of the molecule (Federici 1993; Brar, Verma et al. 2007). The susceptibility of the insect depends on what trypsin-like protease is expressed in the specific insect's gut (OECD 2007).

For many decades microbial products containing *Bacillus thuringiensis* (the organism that produces Cry proteins) have been used to control insect pests on a commercial scale and for home garden applications (Glare and O'Callaghan 2000; Shelton, Zhao et al. 2002). Plants that were genetically engineered to express the Cry1A protein have a history of safe use in the U.S. Since the mid-1990s, corn and cotton lines have been commercialized without substantiated reports of significant deleterious impacts on non-target organisms (OECD 2007; USEPA 2008).

As stated on pp.36 of the submitted petition (08-340-01p) "Over the past 50 years, current use of *Bt* pesticides, including those expressing Cry1Ab, is estimated to be several tons annually. Moreover, Cry1Ab protein is expressed in a number of genetically modified crops that have been approved since 1995 and are currently commercialized. No records of allergenicity in humans and mammals were found associated with *Bt* bacteria (OECD, 2007). In addition, microbial *Bt* biopesticides, including those containing the Cry1Ab protein, have shown no toxic effects in several mammalian toxicity studies (Betz *et al.*, 2000)."

Cry proteins are not expected to adversely affect non-target invertebrates, such as bees, and vertebrate organisms, including birds, mammals and humans, because they do not contain the receptor found in the midgut of target insects. Data provided in the petition (summarized in the Table 30 and 31, pg. 80-81 and data found on pg. 82-83) confirmed that in the mammal, bird, honey bee, above ground arthropod, and soil dwelling invertebrate studies, no observable

adverse effects or differences in survival were noted at for both Cry proteins. The amount of the Cry proteins tested were well above those expected from exposure to the Cry proteins from TwinLink™ cotton planted in the field. The nontarget above-ground arthropods and soil-dwelling invertebrates studied (ladybugs, green lacewings, springtail, daphnia, honeybees, collembola and earthworms) were considered to be representative of the cotton agro-ecosystem.

Although not an endangered or threatened species, *Danaus plexippus* (monarch butterfly) is a species of high conservation interest, and there has been concern that it may be harmed by consuming pollen from transgenic insect-protected cotton and corn. The monarch is susceptible to Cry1Ab (Hellmich, Siegfried et al. 2001) the most common insecticidal protein in transgenic maize and cotton. However, the distribution of the monarch's food plant (*Asclepias syriaca* - common milkweed), the monarch's pattern of migration (Sears, Hellmich et al. 2001), and the lack of cotton pollen movement and adherence to available milkweed (cotton pollen is heavy and sticky) means that very few monarchs are exposed to harmful concentrations of Cry1Ab. TwinLink™ cotton, therefore, poses low risk to monarchs because of minimal hazard of Cry1Ab and Cry2ac and low exposure to Cry protein-containing pollen. TwinLink™ cotton is expected to have no harmful effects on any endangered or threatened species in the U.S.

5. Potential impacts from transferring genetic information from BCS TwinLink™ cotton to organisms with which it cannot interbreed.

APHIS examined the potential for the new genetic material inserted into TwinLink™ cotton to be horizontally transferred to other organisms without sexual reproduction and whether such an event could lead directly or indirectly to disease, damage, injury or harm to plants, including the creation of more virulent pathogens. The horizontal gene transfer between unrelated organisms is one of the most intensively studied fields in the biosciences since 1940, and the issue gained extra attention with the release of transgenic plants into the environment (Dröge, Puhler et al. 1998). Potential risks from stable horizontal gene transfer (HGT) from genetically engineered organisms to another organism without reproduction or human intervention were recently reviewed (Keese 2008). Mechanisms of HGT include conjugation, transformation and transduction, and other diverse mechanisms of DNA and RNA uptake and recombination and rearrangement, most notably through viruses and mobile genetic elements. HGT has been a major contributor to the spread of antibiotic resistance amongst pathogenic bacteria and the emergence of increased virulence in bacteria, eukaryotes and viruses and in the long run has contributed to major transitions in evolution.

a) Potential for Horizontal Gene Transfer to Bacteria or Fungi

TwinLink™ cotton has three bacteria genes. Horizontal gene transfer and expression of DNA from a plant species to other bacterial species is unlikely to occur based on the following observations. Although there are many opportunities for plants to directly interact with fungi and bacteria (e.g. as commensals, symbionts, parasites, pathogens, decomposers, or in the guts of herbivores), there are almost no evolutionary examples of HGT to bacteria from eukaryotes or from plants to fungi (Keese 2008). The only genes likely to be transferred successfully from genetically engineered plants to bacteria are other bacterial genes. Horizontal transfer from and expression in bacteria of the foreign DNA inserted into the nuclear genome of TwinLink™

cotton is unlikely to occur. First, many genomes (or parts thereof) have been sequenced from bacteria that are closely associated with plants including *Agrobacterium* and *Rhizobium* (Kaneko, Nakamura et al. 2000; Wood, Setubal et al. 2001). There is no evidence that these organisms contain genes derived from plants. Second, in cases where review of sequence data implied that horizontal gene transfer occurred, these events are inferred to occur on an evolutionary time scale on the order of millions of years (Koonin, Makarova et al. 2001; Brown 2003). Third, transgene DNA promoters and coding sequences are optimized for plant expression, not prokaryotic bacterial expression. Thus even if horizontal gene transfer occurred, proteins corresponding to the transgenes are not likely to be produced. Fourth, the FDA has evaluated horizontal gene transfer from the use of antibiotic resistance marker genes and concluded that the likelihood of transfer of antibiotic resistance genes from plant genomes to microorganisms in the gastrointestinal tract of humans or animals, or in the environment, is remote (<http://vm.cfsan.fda.gov/~dms/opa-armg.html>).

b) Potential for Horizontal Gene Transfer to Viruses

APHIS also considered whether horizontal transfer of DNA from TwinLink™ cotton to plant viruses was likely to occur and would lead to the creation or selection of a more virulent plant pathogen through recombination with other plant viruses. This issue has been considered before by other science review panels and government regulatory bodies (Keese 2008). The only virus sequences contained within TwinLink™ cotton encode regulatory elements: the cauliflower mosaic virus, the cassava vein mosaic virus and the subterranean clover stunt virus. Regulatory elements such as promoters and terminators have not been implicated in viral recombination.

Therefore, APHIS concludes that horizontal gene transfer is unlikely to occur and thus poses no significant environmental or plant pest risk. Finally, under natural conditions; no transfer of an intact functional gene has been demonstrated to date (Miki and McHugh 2004). Therefore APHIS concludes that horizontal gene transfer is unlikely to occur and thus poses no significant environmental or plant pest risk.

D. Conclusion

APHIS has reviewed and conducted a plant pest risk assessment on BCS TwinLink™ cotton. Due to the lack of plant pest risk from the inserted genetic material, the lack of weediness characteristics of BCS TwinLink™ cotton, the lack of atypical responses to disease or plant pests in the field, the lack of deleterious effects on non-targets or beneficial organisms in the agro-ecosystem, and the lack of horizontal gene transfer, APHIS concludes that BCS TwinLink™ cotton is unlikely to pose a plant pest risk.

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