United States
Department of
Agriculture

Marketing and
Regulatory
Programs

Animal and
Plant Health
Inspection
Service

Determination of Non-regulated Status for Glyphosate-Tolerant (GlyTol™) Cotton, *Gossypium hirsutum*, event GHB614

Petition 06-332-01p

Environmental Assessment
16 May 2008

Determination of Non-regulated Status for Glyphosate-Tolerant (GlyTol™) Cotton, *Gossypium hirsutum*, event GHB614

Petition 06-332-01p

Environmental Assessment
16 May 2008

Agency Contact:
Cynthia Eck, Document Control Officer
USDA, APHIS, BRS
4700 River Road, Unit 146
Riverdale, MD 20737-1237
Phone: (301) 734-0667
Fax: (301) 734-8669
Email: Cynthia.A.Eck@usda.gov

Table of Contents

I. Purpose & Need

The Animal and Plant Health Inspection Service of the United States Department of Agriculture (USDA-APHIS), Biotechnology Regulatory Service (BRS) protects America's agriculture and environment using a dynamic and science-based regulatory framework that allows for the safe development and use of genetically engineered plants.

APHIS regulations (7 CFR part 340) regulate the introduction (importation, interstate movement or release into the environment) of certain genetically engineered plants or plant pests. An organism is no longer subject to the regulatory requirements of 7 CFR part 340 when it is demonstrated that it does not present a plant pest risk. A genetically engineered organism is considered a regulated article if the donor organism, recipient organism, vector, or vector agent used in engineering the organism belongs to one of the taxa listed in the regulation (7 CFR 340.2) and is also considered a plant pest.

Under these regulations, a petitioner may file an application requesting that APHIS review the regulated article and evaluate submitted data and determine that a particular regulated article does not present a plant pest and, therefore, should no longer be regulated under 7 CFR 340.6 "Petition for Determination of Nonregulated Status." The petitioner is required to provide certain information which the agency uses to determine whether the regulated article is unlikely to present a greater plant pest risk than the unmodified organism. If, based on the information, the agency determines that the article is unlikely to pose a plant pest risk, the article must be granted deregulated status. Under APHIS regulations 7 CFR part 340, the receipt of a petition application to introduce a genetically engineered organism requires a response from the Administrator:

> *Administrative action on a petition*. The Administrator shall furnish a response to each petitioner within 180 days of receipt of the petition. The response will either: (i) Approve the petition in whole or in part in which case the Administrator shall concurrently take appropriate action (publication of a document in the FEDERAL REGISTER amending 340.2 of this part; or (ii) deny the petition in whole or part. 7 CFR part 340.5 (c3).

USDA-APHIS, BRS has prepared an Environmental Assessment (EA) in response to a petition (APHIS Number 06-332-01p, received on November 20, 2006) submitted by Bayer CropScience (BCS) for a determination of non-regulated status for genetically engineered (GE) GlyTol™ cotton *(Gossypium hirsutum*, event GHB614) developed to express tolerance to the herbicide, glyphosate[1]. GlyTol™ cotton is currently a regulated article under USDA regulations at 7 CFR part 340, and as such, interstate movements, importations, and field tests of the transformed cotton have been conducted under notifications issued by APHIS. BCS has submitted a petition application to APHIS requesting a determination that GlyTol™ cotton does not present a plant pest risk, and therefore, GlyTol™ cotton and its progeny derived from crosses with other non-regulated cotton should no longer be regulated articles under these APHIS regulations.

BCS has developed GlyTol™ cotton (event GHB614) as an alternative glyphosate-tolerant cotton product to Monsanto's Roundup Ready® cotton that has provided an established weed management tool to producers since its deregulation in July 1995. APHIS has reviewed the data

[1] Glyphosate tolerant and glyphosate resistant are used interchangeably in this document.

supplied by the petitioner and current scientific literature, and found GlyTol™ cotton to be a no greater plant pest risk than the unmodified organism. APHIS reviews the GE plant for known and potential differences from the original plant. These include disease and pest susceptibilities, expression of the gene product, weediness of the GE plant, impact on the weediness of another other plant with which it can interbreed, agricultural or cultivation practices and transfer of genetic information to organisms with which it cannot interbreed. Information on cotton in general, the weediness of cotton, gene flow and plant pest risks is discussed in this document in Appendix A. If APHIS had found that GlyTol™ cotton demonstrated greater plant pest risks after reviewing the data given by the applicant, APHIS would have chosen to keep GlyTol™ cotton a regulated article (see No Action Alternative, Section III).

In accordance with APHIS procedures for implementing the National Environmental Policy Act of 1969 (NEPA, 7 CFR part 372), this EA has been prepared for GlyTol™ cotton in order to specifically evaluate how the proposed action and alternatives described in the following section, if implemented, may affect the quality of the human environment.

II. Affected Environment

A. Background

1. Cotton

The genus *Gossypium*, a member of the Malvaceae family, consists of 39 species; four of which are generally cultivated in the world (Fryxell 1979). The most commonly cultivated species in the United States is *G. hirsutum* (common name, Upland cotton); comprising 97% of the U.S. cotton crop (www.ers.usda.gov) and is the subject of this EA. Limited amounts of *G. barbadense* are cultivated in Hawaii. Other cultivated species around the world are *G. arboreum*, *G. barbadense*, and *G. herbaceum*. There are two wild species of cotton found in the United States; *G. thurberi* and *G. tomentosum*, of Arizona and Hawaii, respectively.

Cotton is the leading fiber crop in the United States as well as the world. It is the leading 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 is a perennial plant cultivated as an annual, and is grown in the United States in just 18 states, from Virginia southward and westward to California; in an area often referred to as the Cotton Belt (McGregor 1976). Cotton is more limited geographically than any other major crop in the United States because it can be grown only in those regions in which there are more than 180 frost-free days per year (those states in the Cotton Belt). Because of its limited geographic production area, this EA will focus its review to the major cotton producing states of Texas, Georgia, Arkansas, Mississippi, North Carolina, Tennessee, Louisiana and California (www.cottonusa.org).

In the 2005-2006 production years, the United States grew 13.8 million acres of cotton (USDA-FAS 2006). According to Cotton Council International (www.cottonusa.org) the major cotton-producing states were Texas (8,440 thousand bales), Georgia (2,140 thousand bales), Arkansas (2,202 thousand bales), Mississippi (2,147 thousand bales), North Carolina (1,437 thousand bales), Tennessee (1,112 thousand bales), Louisiana (1,098 thousand bales) and California (1,065 thousand bales). There were 85 million acres of cotton planted worldwide in 2005-2006, producing a total of 114 million bales of cotton (USDA-FAS 2006). Of the 2007 acreage planted in the United States, it has been estimated that 87% of cotton is genetically-engineered for herbicide-tolerance, insect-tolerance or both (http://www.ers.usda.gov/Data/BiotechCrops/adoption.htm). Cotton Council International (www.cottonusa.org) estimates the use of genetically-engineered (for all traits) cotton in the U.S. to be as much as 95.5%. The remaining percentage of cotton grown is traditional cotton seed with a small (<0.5%) percentage of cotton grown organically. More information on cotton production can be found in the Selected Resource Materials section at the end of this document. The estimates by USDA-FAS and Cotton Council International were based on surveys completed by participating farmers from the areas of interest.

2. Weed Competition and Control in Cotton

Cotton is more susceptible to weeds than soybeans or corn because it is easily out-grown during its early season growth. Weeds also interfere with harvest equipment and can cause lint staining[2], all leading to major crop and economic losses. The key to successful cotton production is a weed management program which includes crop rotation, herbicide application and weed surveillance and monitoring.

Herbicide-tolerant crops, such as BCS' GlyTol™ (glyphosate-tolerant) cotton, are developed to survive application of herbicides (in this case glyphosate) that previously would have destroyed the crop along with the targeted weeds. These herbicide-tolerant crops are providing farmers with a broader variety of options for effective and economically affordable weed control. Based on USDA survey data, plantings of herbicide-tolerant cotton expanded from 14 percent of U.S. acreage in 1997 to 56 percent in 2001, 65 percent in 2006, and 87% in 2007 (http://www.ers.usda.gov/Data/BiotechCrops/adoption.htm).

There are many weeds found in cotton production fields that naturally resist glyphosate or are difficult to control by only applying glyphosate. Bermuda grass, dove weed, Florida pusley, hemp sesbania, morning glory, nutsedge, tropical spiderwort, horseweed and palmer amaranth are examples of naturally resistant weeds (Weed Management in Cotton, http://commodities.caes.uga.edu/fieldcrops/cotton). Because of this natural resistance to glyphosate, cotton growers use a variety of herbicides (not just glyphosate) for successful cotton production. Table 1 contains a list of common herbicides used in cotton production. Table 2 shows typical herbicide strategies for herbicide use on cotton; whether the crop is transgenic for glyphosate-resistance or non-transgenic, and whether there have been glyphosate-resistant weeds surveyed within the cotton acreage (active ingredients and manufacturer can be found in Table 1). The mixing of herbicides is a strategy used by the producer to sustain good weed management. Growers need to consult with their local agricultural extension agent to gain an understanding of what herbicide regime is appropriate in their area. It is the continued and exclusive use of one herbicide that selects for the resistant weeds that creates a problem in any crop production. There are many websites that discuss weed management in herbicide-tolerant crops, e.g. http://www.weedresistancemanagement.com/layout/default.asp as well as the previously mentioned University of Georgia website (http://commodities.caes.uga.edu/fieldcrops).

Table 1. Trademark Herbicides and Manufacturers

Registered Trademark	Active Ingredient(s)	Manufacturer[1]
Clarity	Dicamba diglycolamine salt	BASF
2,4 D-Express	2,4-dichlorophenoxy acetic acid + tribenuron methyl + dicamba	DuPont
Harmony Extra	Thifensulfuron methyl + thibenuron methyl	DuPont
Valor	Flumioxazin	Valent
Ignite	Glufosinate-ammonium	Bayer CropScience
Gramoxone	Paraquat	Syngenta
Direx	Diuron	DuPont

[2] Lint staining refers to the coloration of the raw cotton fiber. Color deterioration affects the ability of cotton fibers to absorb and hold dyes and finishes, thus reducing its value.

Caparol	Prometryn	Syngenta
Prowl	Pendimethalin	BASF
MSMA plus S	Monosodium acid methanearsonate	Dow
Treflan	Trifluralin	Dow
Cotoran	Fluometuron	Makhteshim-agan
Reflex	Fomesafen	Syngenta
Staple	Pyrithiobac sodium	DuPont
Dual Magnum	S-metolachlor	Syngenta
Sequence	Glyphosate and S-metolachlor	Syngenta
Suprend	Trifloxysulfuron sodium + prometryn	Syngenta
Layby Pro	Diuron + Linuron	DuPont

[1]There can be more than one manufacturer for some older products, depending on formulation.

Table 2.[1] Weed Control Programs for Managing Glyphosate- and ALS-Resistant Weeds in Cotton.[2,3]

Cotton Variety	Glyphosate Resistance Suspected	ALS[4] Resistance Suspected	Preplant Incorporated or Preemergence	Postemergence 1- to 4-leaf cotton	Layby Options (Palmer < 3 in.)
Any	Yes or No	Yes or No	--------------------**Burndown Options**-------------------- Glyphosate + Clarity, 2,4-D, Express, Harmony Extra, Prowl or Valor; Ignite + 2,4-D or Clarity; Gramoxone + Direx or Caparol		
Roundup Ready® (Monsanto's glyphosate tolerant variety)	No	Yes	Prowl or Treflan PPI or Prowl, Cotoran, or Reflex PRE or Prowl + Cotoran	Glyphosate or Glyphosate + Dual Magnum (or Sequence)	MSMA or Glyphosate + Caparol, Direx, Suprend, or Valor or Layby Pro or Layby Pro + MSMA
***Roundup Ready® (Monsanto's glyphosate tolerant variety)**	Yes	No	Prowl or Treflan PPI followed by Cotoran, Reflex or Staple PRE or Prowl + Reflex or + Staple[5] PRE	No Palmer emerged: Glyphosate + Dual Magnum (or Sequence) as needed	MSMA + Caparol, Direx, Suprend, or Valor or Layby Pro or Layby Pro + MSMA
				Palmer < 2 in: Glyphosate + Staple	Same as Above
Non-Transgenic	Yes or No	Yes	Prowl or Treflan PPI followed by Cotoran or Reflex PRE or Prowl + Cotoran or Reflex PRE	MSMA + Cotoran, or Caparol, only as a directed application	MSMA + Caparol MSMA + Direx MSMA + Layby Pro MSMA + Valor

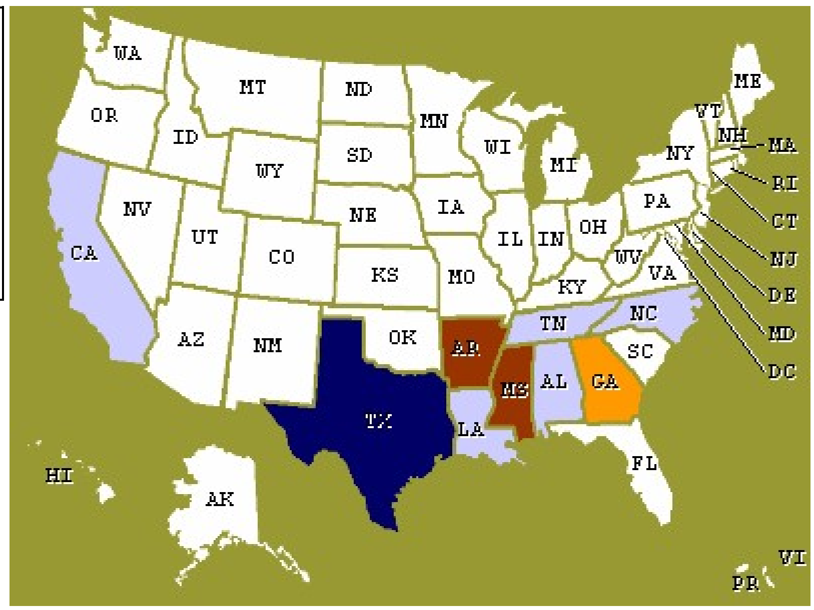
[1]This table from Burgos, et al. (Burgos, Culpepper et al. 2006) was modified to exclude the Liberty Link cotton product. All products are registered trademarks of respective companies.
[2]For glyphosate-resistant Palmer amaranth, hooded sprays with paraquat mixtures, cultivation, and/or hand weeding will often be required.
[3]Herbicide labels vary among regions. Follow labels for soils and regions. Note that in Texas west of I-35, Reflex (Syngenta) cannot be used preemergence, and Suprend (Syngenta) cannot be used postemergence – use as directed.
[4]ALS = Acetolactate Synthase Inhibitors
[5]Limit Staple (DuPont) use to once per season

3. Glyphosate Use on Cotton in the Cotton Belt

The use of the herbicide, glyphosate, is directly proportional to the amount of cotton each state produces (Figure 1). As of May, 2008, the year 2005 is the latest data available for the major cotton-producing states from the National Agricultural Statistics Service (NASS) Agricultural Chemical Use Database. The data shows that Texas uses the most glyphosate followed by Arkansas and Mississippi, Georgia, North Carolina, Tennessee, Louisiana and California. This data mirrors the amounts of cotton produced in these states as reported by Cotton Council International (www.cottonusa.org).

Figure 1. 2005 Glyphosate Use in the Major Cotton-producing States

Color	Equal To/ Greater Than	But Less Than
	No Data	
	278.0	1141.0
	1141.0	2004.0
	2004.0	2867.0
	2867.0	3730.0



4. Glyphosate Tolerant Plants and Effects on Humans, Animals and Plants

The glyphosate herbicide (N-phosphonomethyl-glycine) is registered with the Environmental Protection Agency (EPA) for non-selective weed control on both non-food use and food use plants. Glyphosate tolerance in a plant is made by mutating the *EPSPS* gene. All plants contain the *EPSPS* gene. This gene makes enzyme, 5-enolpyruvyl-3-phosphoshikimate acid synthase (EPSPS). Without this enzyme, the plant cannot process aromatic amino acids (phenylalanine, tryptophan, and tyrosine and some secondary metabolites) and the plant dies. The herbicide glyphosate functions due to its resemblance of the structure of the substrate for EPSPS enzyme and thereby competing with this substrate for the enzyme's active site, thus preventing the synthesis of aromatic amino acids[3] (and killing the plant).

[3] A more comprehensive explanation of the mechanism for glyphosate can be found in Appendix B.

Animals (including humans) do not have this EPSPS enzyme and obtain aromatic amino acids from their diet. Consequently, all animals (including humans) are naturally exposed to sources of EPSPS by ingesting plant materials.

GM feed is digested by animals in the same way as conventional feed. Food from animals fed on authorized GM crops is considered to be as safe as food from animals fed on non-GM crops. In a statement published on 19 July 2007, European Food Safety Authority (EFSA) advised that 'Biologically active genes and proteins are common constituents of food and feed in varying amounts. After ingestion, a rapid degradation into short DNA or peptide fragments is observed in the gastrointestinal tract of animals and humans. To date, a large number of experimental studies with livestock have shown that recombinant DNA fragments or proteins derived from GM plants have not been detected in tissues, fluids or edible products of farm animals like broilers, cattle, pigs or quails' (EFSA 2007).

III. Alternatives

This EA analyzes the potential environmental consequences of a proposal to deregulate GlyTol™ cotton. Two alternatives are considered in this EA: (1) no action, and (2) to grant the deregulated status for GlyTol™ cotton. One other alternative was considered and dismissed: the approval of the petition with geographic restrictions (approval of the petition, in part). This alternative is only available when supporting data is not sufficient to determine that GlyTol™ cotton is unlikely to present a greater plant pest risk than the unmodified organism in certain geographical areas. The analysis provided in the risk assessment (Appendix A) shows that there was sufficient data to determine that GlyTol™ cotton does not pose a pest risk. APHIS was not able to envision a scenario upon which mitigation of any plant pest risk posed by this cotton would be necessary. The company has provided enough data describing GlyTol™ cotton agronomic traits and there was no evidence to suggest that there is a greater risk of being a plant pest in a specific geographic location.

A. No Action

Under the no action alternative, APHIS would not deregulate GlyTol™ cotton. As such, GlyTol™ cotton would not be available to the general public in the marketplace as a choice of available glyphosate tolerant cotton. Bayer CropScience would have to continue to request permits and notifications for field tests of GlyTol™ cotton. APHIS' review of the petition together with scientific literature has lead to a finding that there is sufficient evidence to demonstrate the lack of a plant pest risk. Choosing the no action alternative would not meet the purpose and need of this action because it does not allow for the safe development and use of genetically engineered plants.

B. Preferred Alternative

Under the preferred alternative, APHIS would grant the petition for deregulation status for GlyTol™ cotton. Under this alternative, GlyTol™ cotton (event GHB614) would no longer be a regulated article under the regulations at 7 CFR part 340. Permits issued or notifications acknowledged by APHIS would no longer be required for introductions of GlyTol™ cotton derived from these events. This product would be used as an alternative market choice that provides an established weed management tool to producers. This alternative would meet the purpose to allow for the safe development and use of genetically engineered plants by deregulating GlyTol™ cotton which has been proven not pose a plant pest risk (see Appendix A).

IV. Environmental Consequences

A. No Action

Under the no action alternative, the product would not be available for the consumer in the marketplace. GlyTol™ cotton would continue to be regulated under permits and notifications by APHIS, BRS.

1. Glyphosate Use on Cotton in the Cotton Belt

If APHIS does not approve the deregulation of GlyTol™ cotton, genetically engineered cotton that is glyphosate-tolerant would continue to be planted in the cotton belt with the continuing trends in herbicide use. The glyphosate tolerant cotton from Monsanto (Roundup Ready® cotton) would continue to be planted. Table 3 shows the herbicide usage trends in cotton since 1997. APHIS believes the trends for glyphosate usage will continue to increase even if GlyTol™ cotton is not deregulated because its sister product (Roundup Ready® cotton) would continue to dominate the market as it has for the past 11 years. Denying the deregulation of GlyTol™ cotton would not meet APHIS, BRS' mission to protect America's agriculture and environment using a dynamic and science-based regulatory framework that allows for the safe development and use of genetically engineered organisms based on the designation that this article does not pose a plant pest risk (see Appendix A).

Table 3. Herbicide Usage Trends in Cotton from 1997 – 2005

Herbicide	1997		2001		2003		2005	
	%Area Treated	Active Ingredient 1000 lb/yr	%Area Treated	Active Ingredient 1000 lb/yr	%Area Treated	Active Ingredient 1000 lbs/yr	%Area Treated	Active Ingredient 1000 lb/yr
Glyphosate	14	1,542	57	8,514	69	12,635	71	14,112
Trifluralin	55	5,461	30	3,066	39	4,156	32	3,522
Diuron	12	883	26	1,545	28	1,738	27	1,707
Pendimethalin	28	2,491	16	1,651	20	1,813	12	1,211
Pyrithiobac-sodium	23	171	10	85	12	124	9	50
Prometryn	19	1,669	12	1,292	11	1,175	7	669
Fluometuron	44	4,847	10	977	8	755	5	487
MSMA/DSMA	33	4,899	11	1,834	7	1,157	6	937
Metolachlor [a]	5	735	4	419	5	591	6	847
Clomazone	8	500	NS[c]	NS	<0.5	16	<0.5	12
Clethodim	2	37	2	28	<0.5	14	1	19
States surveyed[b]	AL, AZ, AR, CA, GA, LA, MS, MO, NC, SC, TN, TX		AL, AZ, AR, CA, GA, LA, MS, MO, NC, SC, TN, TX		AL, AZ, AR, CA, GA, LA, MS, MO, NC, SC, TN, TX		AL, AR, CA, GA, LA, MS, NC, SC, TN, TX	
Acreage represented in survey[b]	13,075,000 (96%)		12,680,000 (93%)		12,795,000 (90%)		12,425,000 (89%)	
Total planted cotton acreage[d]	13,898,000		15,768,500		13,479,600		14,245,200	

[a]Includes both racemic and S-forms of metolachlor.
[b]USDA-NASS, 2007. Agricultural Chemical Usage Database. (http://www.pestmanagement.info/nass/app_usage.cfm)
[c]NS = not surveyed
[d]USDA-NASS, 2007. Cotton, National Statistics Database. (http://www.nass.usda.gov/QuickStats/index2.jsp)

2. Glyphosate Tolerant Cotton and its Effects on Humans, Animals and Plants

The first glyphosate tolerant cotton to be deregulated by APHIS was Roundup Ready® cotton lines 1445 and 1698, which were submitted as Petition 95-045-01p by Monsanto and deregulated by APHIS in July, 1995. This event was the result of incorporating the gene coding for the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (*EPSPS*) gene derived from *Agrobacterium* sp. strain CP4, a common soil bacterium. For the past 11 years, utilization of a glyphosate herbicide plus Roundup Ready® cotton has provided another tool to use in weed control and encouraged the use of conservation-tillage[4] (Brookes and Barfoot 2006). In 1997, Monsanto submitted a petition to deregulate glyphosate-resistant *Zea mays* (corn) with a modified corn EPSPS protein (mEPSPS) (Event GA21; APHIS petition number 97-099-01). This product was deregulated by APHIS in 1998 and approved by Canada for food use in October 1999, demonstrating a 9 year history of safe usage for the corn EPSPS protein[5].

Glyphosate tolerant cotton has been on the market since its deregulation in 1997 after extensive testing by Monsanto and evaluation by APHIS, EPA and FDA. The use of glyphosate tolerant cotton for the past 11 years has continually demonstrated the safety and effectiveness of this weed management tool and has had no known adverse effects on animals (including humans). Choosing the No Action alternative and preventing the deregulation of GlyTol™ cotton would not affect the history of safe use of glyphosate tolerant cotton and Roundup Ready® cotton would continue to dominate the marketplace.

B. Preferred Alternative

Under this alternative, GlyTol™ cotton would be deregulated by APHIS and allowed to compete with its sister product in the marketplace. This alternative would meet APHIS, BRS' mission to protect America's agriculture and environment using a dynamic and science-based regulatory framework that allows for the safe development and use of genetically engineered organisms based on the designation that this article does not pose a plant pest risk (see Appendix A).

1. Glyphosate Use on Cotton in the Cotton Belt

Widespread use of GlyTol™ cotton is not expected to have an impact on typical cotton production since its sister product has been in use for the past 11 years as a successful weed management tool. Since glyphosate-resistant cotton has been on the market so long, it is believed that market saturation has already occurred with this type of product (USDA-NASS 2007). According to the 2005 surveys (USDA-NASS 2007), the market trend is for a product that contains both insect and herbicide resistance in cotton.

The introduction of this product into the market will allow consumers a choice in brand names and is not expected to increase cotton acreage. Other than the glyphosate-resistant gene, the GlyTol™ cotton plant will not produce any other substance that is not normally produced by cotton plants, nor is the composition of the cotton boll produced by these plants different from unmodified cotton. Therefore, APHIS does not expect accumulation of a novel substance in soil,

---

[4] Conservation-tillage refers to growing crops with minimal cultivation of soil. New crops are planted into the small strips of tilled soil. Weeds are controlled with cover crops or herbicides rather than plowing or disking plant remains from previous crops into the top soil.

[5] More information on glyphosate and the *EPSPS* gene can be found in Appendix B.

nor does APHIS expect impacts on organisms living in and around cotton production areas because of exposure to GlyTol™ cotton plants.

2. GlyTol™ Cotton and Its Effects on Humans, Animals and Plants

GlyTol™ cotton contains a mutated corn *EPSPS* and its protein differs from the native protein by only two amino acids (Table 8, p.40 of submitted petition). The 2mEPSPS protein has no amino acid sequence similarity to known allergens or toxins. Bayer CropScience has conducted a detailed safety evaluation on the Codex Alimentarius Commission's (Codex 2003) database that included homology searches of allergen databases, *in vitro* digestibility assay and acute toxicity testing in mice.

APHIS authorized the first field testing of the BCS GlyTol™ cotton plants starting in 2002 and they have been field tested in the United States under the APHIS authorization numbers noted in the petition 06-332-01p, Appendix 1 pages 86-105. GlyTol™ cotton plants have been evaluated extensively to confirm that they exhibit the desired agronomic characteristics, that tolerance to glyphosate is stable under field conditions and that they do not present a plant pest risk (petition 06-332-01p, p.126-132). The field tests have been conducted in agricultural settings under physical and reproductive confinement conditions. Plant pest risks are discussed in this EA in Appendix A.

APHIS considered the agronomic data that was submitted by the developer (Section VIII, pg 43-63 of the petition), as well as the cooperating growers' visual field observations to determine if there were changes to non-target species associated with glyphosate-resistant cotton. A comparison of the compositional analysis on the plants containing 2mEPSPS protein with their non-transgenic counterparts indicated no significant changes in the overall gossypol content of the plants or anti-nutrient levels between GlyTol™ cotton event GHB614 and the non-transgenic counterpart (Section VIII.I of the petition). Gossypol is a natural toxin present in cotton, and in large amounts is toxic to livestock and humans. For this reason, there are limits to the amount of cotton meal given to livestock and cotton seed oil must be refined before food use. The GlyTol™ cotton plants do not express additional proteins, natural toxicants, pheromones, hormones, etc. that could directly or indirectly result in killing or interfering with the normal growth, development, or behavior of a non-target species. Cooperative growers did not report any visual differences in bird, insect, or other non-target populations between GlyTol™ cotton event GHB614 and its non-transgenic counterpart. Field observations are summarized in termination reports located in Appendix I of the petition.

APHIS further considered the biology of the GlyTol™ cotton with respect to its potential to affect non-target organisms such as beneficial insects (including pollinators such as bees), and biocontrol organisms. No differences between the transgenic and non-transgenic cotton in the flower morphology or time to bloom were found. Additionally the 2mEPSPS protein is expressed at a very low level in cotton pollen (0.16 μg/g fresh weight; p37 in submitted petition). Because no other changes in the bloom pattern or toxicity of the cotton plant were found, it is not anticipated that pollinating species, primarily insects, would be impacted by GlyTol™ cotton. No differences in the development or morphology between the transgenic and non-transgenic cotton lines were found which would indicate any adverse impact on foliar beneficial insects. Because no changes in the overall gossypol content and anti-nutrient levels of the plant were detected, it is not anticipated that the GlyTol™ cotton event GHB614 has a higher degree of risk from the toxin gossypol than non-transformed cotton.

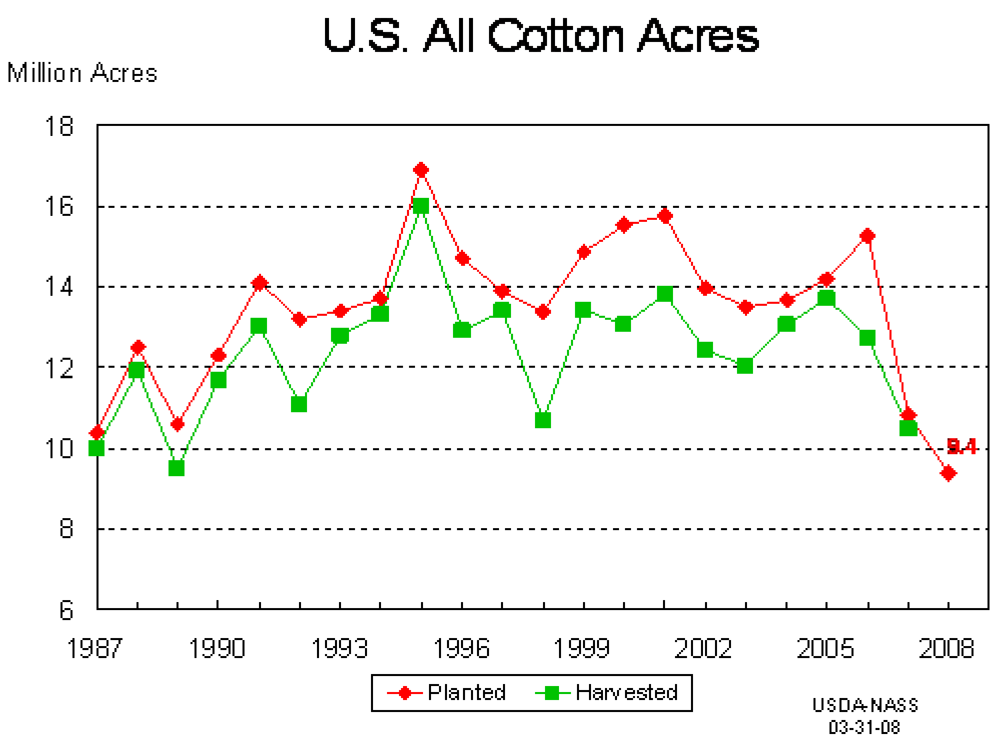
C. Cumulative Effects

APHIS considered whether the proposed action could lead to cumulative impacts, when considered in light of other past, present, and reasonably foreseeable future actions, regardless of what agency or person undertakes such actions.

1. Cotton

Typically, cotton production occurs on land that has been dedicated to agricultural use for greater than three years and has 180 frost-free days. As with most cotton production, it is seasonally rotated with soybeans, corn or cereal crops and would normally include the use of resources to limit the growth of weeds, limit the potential impact caused by insects, animals or disease, and to maximize production (Endrizzi, Turcotte et al. 1984). In 2007, 87% of the cotton acreage was planted to all GE varieties (this includes herbicide tolerant and insect tolerant), about 91% of the soybean acreage was planted to all GE varieties, and about 73% of the corn acreage was planted to all GE varieties (USDA-NASS 2007). Currently, there is no GE wheat or barley available on the market. Deregulation of GlyTol™ cotton is not a product expected to have any additive effects by increasing cotton acreage, but rather will provide farmers and other consumers of cotton seeds with an additional choice of GE cotton product. Due to the planting of higher paying crops destined for biofuels and the lower price of cotton, the total cotton acreage in 2007 decreased 28% from 2006 (Figure 2, http://www.nass.usda.gov/Charts_and_Maps/Field_Crops/cotnac.asp).

Figure 2. U.S. Cotton Acreage



2. Weed Competition and Control

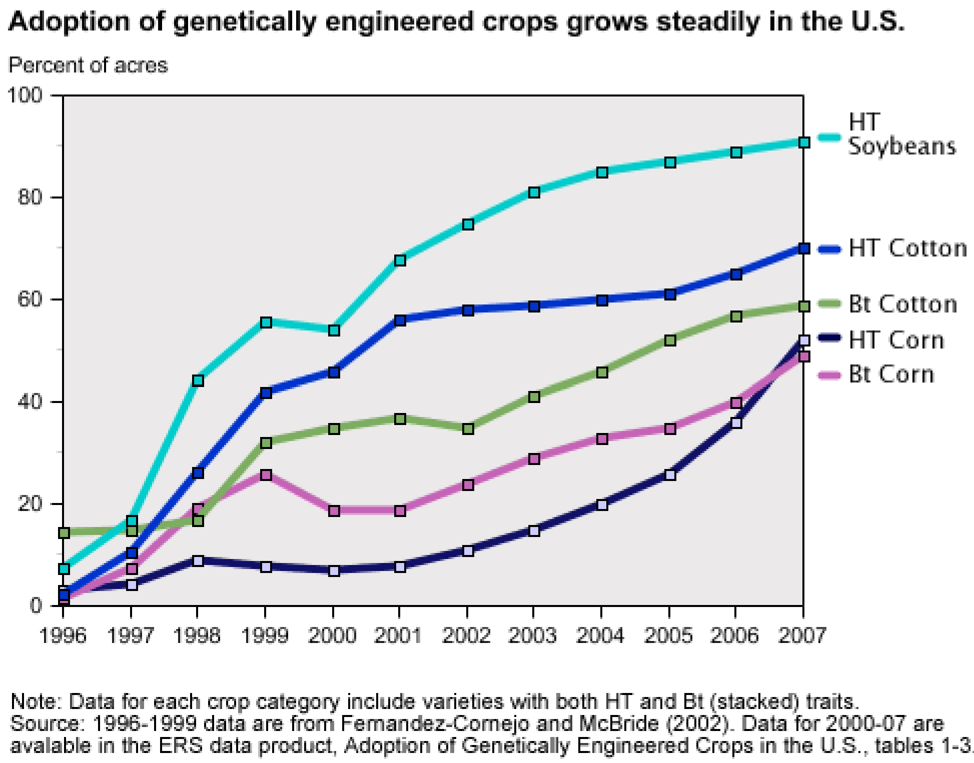
Along with the increasing adoption of these GE crops there is an increasing use of the herbicide glyphosate and the associated decreasing use of other herbicides (see Table 3 for an example how cotton has been affected). Compared to the herbicides it replaces, the glyphosate used on these crops is less toxic to humans and not as likely to persist in the environment as the herbicides it replaces (IPCS 1994; USDA-ERS 2006). The total amount of glyphosate used on GE cotton is not expected to increase with the deregulation of GlyTol™ cotton because the product provides consumers with a choice of GE cotton seed to purchase, and the adoption of glyphosate-tolerant cotton is believed have reached its maximum market potential (USDA-NASS 2007). APHIS, BRS does not foresee any increased glyphosate use by the addition of GlyTol™ cotton to the market.

As discussed above in the background information, economical weed control in cotton needs an integrated weed management (IWM) strategy to minimize the development of herbicide-resistant weeds. Continuous use of one product to control weeds will select for weed types that are not affected by that one product. Effective management of competitive weeds in cotton requires the use of many tools that include cultural, mechanical, biological and chemical means. There are many websites on IWM (two of which are mentioned above) that provide easy to follow information on how to use glyphosate-resistant cotton, along with other management tools, to control weeds economically.

3. Glyphosate Use on All Major GE Crops in the Cotton Belt

According to data supplied by the USDA Economic Research Service (ERS), U.S. farmers have adopted genetically engineered (GE) crops widely since their introduction in 1996. Soybeans and cotton genetically engineered with herbicide-tolerant (HT) traits have been the most widely and rapidly adopted GE crops in the U.S., followed by insect-resistant cotton and corn (Figure 3, Bt refers to GE protein that is expressed in these insect-resistance crops isolated from *Bacillus thuringiensis*).

Figure 3



Herbicide-tolerant crops (all herbicide tolerant traits, not just glyphosate tolerance) provide farmers with a broader variety of options for effective weed control. Based on USDA-ERS survey data, HT soybeans went from 17 percent of U.S. soybean acreage in 1997 to 68 percent in 2001 and 91 percent in 2007. Plantings of HT cotton expanded from 10 percent of U.S. acreage in 1997 to 56 percent in 2001 and 70 percent in 2007. The adoption of HT corn, which had been slower in previous years, has accelerated, reaching 52 percent of U.S. corn acreage in 2007 (USDA-ERS 2006).

Looking at data from 1997 (a year after adoption of GE crops) until 2006 (most recent data available through National Agricultural Statistics Service, http://www.pestmanagement.info/nass/app_usage.cfm), total herbicide use on corn, cotton and soybeans in the U.S. have not shown dramatic increases or decreases; however, glyphosate use has increased during that time (Tables 5 and 6, respectively).

Table 5. Total Herbicide Usage Trends for Corn, Cotton and Soybean from 1997 – 2006

	Herbicides – Total Active Ingredient x1000 lbs/year									
Crop	**1997**	**1998**	**1999**	**2000**	**2001**	**2002**	**2003**	**2004**	**2005**	**2006**
Corn	164051	177012	154059	153464	157239	95777	149136	NA	157575	NA
Cotton	27611	22206	25006	26554	NA	21098	25542	NA	25733	NA
Soybean	78207	71437	70729	75164	50464	86742	NA	70828	77187	NA

NA = data not available

Table 6. Glyphosate Usage Trends for Corn, Cotton and Soybean from 1997 – 2006

	Glyphosate – Total Active Ingredient (x1000 lbs/year)									
Crop	**1997**	**1998**	**1999**	**2000**	**2001**	**2002**	**2003**	**2004**	**2005**	**2006**
Corn	1429	2601	4162	4438	6868	3307	11913	NA	NA	NA
Cotton	1542	3726	5122	9529	8514	NA	12635	NA	14112	NA
Soybean	14915	28123	38447	41847	32806	59962	NA	57701	NA	88903

NA = data not available

The increased use of glyphosate has been a trend in all major crops due to its low cost and low toxicity to applicators (Sankula, Marmon et al. 2005). APHIS, BRS does not believe that glyphosate use on any rotated crops within the Cotton Belt will be impacted by the deregulation of GlyTol™ cotton.

There is an increased trend for the use of the stacked GE cotton traits that contain both insect-tolerant and herbicide-tolerant genes over purely herbicide-tolerant cotton (USDA-ERS 2006; USDA-NASS 2007). By itself, GlyTol™ cotton is not expected to increase glyphosate use as it will exist as a consumer choice in a market that has already reached its saturation (USDA-NASS 2007). While the use of stacked traits in GE cotton (insect- and herbicide-tolerance) is showing an increase, the adoption rates of a purely herbicide-tolerant or insect-tolerant cotton product use is static or declining (USDA-ERS 2006). It is likely that GlyTol™ cotton could be conventionally bred to an insect-tolerant cotton variety in the future. There is the potential that glyphosate use could increase as much as 13% based on the current adoption rates of stacked gene constructs that contain both insect- and herbicide-tolerant GE cotton. These trends will continue to occur in the future whether GlyTol™ cotton is granted deregulation status or not. GlyTol™ cotton's contribution to these continuing trends will be the addition of a consumer choice.

4. Glyphosate Tolerant Cotton and Effects on Humans, Animals and Plants

Data supplied by the applicant, including the results of 11 years of glyphosate-resistant cotton already on the market, suggests that GlyTol™ cotton has not had observable or measurable impacts on the ecosystems in which it has been allowed to grow. This data can be found in the Section IX of the petition application. Another source evaluating the minimal environmental impacts of glyphosate can be found in the International Programme on Chemical Safety on Glyphosate (IPCS 1994).

Currently, APHIS, BRS does not have any other herbicide tolerant cotton applications for deregulation. APHIS, BRS does have an insect-tolerant cotton application for deregulation by another company (http://www.aphis.usda.gov/brs/not_reg.html). The deregulation of BCS' GlyTol™ cotton is not dependent upon the deregulation of the insect-tolerant cotton.

Based upon available information, APHIS has determined that there are no past, present, or reasonably foreseeable actions that would aggregate with effects of the proposed action to create cumulative impacts or reduce the long-term productivity or sustainability of any of the resources (soil, water, ecosystem quality, biodiversity, etc.) associated with the ecosystem in which GlyTol™ cotton is planted. No resources will be impacted due to cumulative impacts resulting from the proposed action.

5. Other Authorities

U.S. Environmental Protection Agency (EPA) and Food and Drug Administration (FDA) Regulatory Authorities

In 1986, the Federal Government's Office of Science and Technology Policy (OSTP) published a policy document known as the Coordinated Framework for the Regulation of Biotechnology. This document specifies three Federal agencies that are responsible for regulating biotechnology in the United States: the U.S. Department of Agriculture's (USDA) Animal and Plant Health Inspection Service (APHIS), the Environmental Protection Agency (EPA), and the U.S. Department of Health and Human Services' Food and Drug Administration (FDA). Products are regulated according to their intended use, and some products are regulated by more than one agency. Together, these agencies ensure that the products of modern biotechnology are safe to grow, safe to eat, and safe for the environment. USDA, EPA, and FDA apply regulations to biotechnology that are based on the specific nature of each GE organism.

The U.S. Environmental Protection Agency (EPA) is responsible for the regulation of pesticides under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), as amended (7 U.S.C. 136 *et seq.*). FIFRA requires that all pesticides, including herbicides and GE biopesticide products, be registered prior to distribution or sale, unless exempt by EPA regulation. In order to be registered as a pesticide under FIFRA, it must be demonstrated that when used with common practices, a pesticide will not cause unreasonable adverse effects in the environment. Under the Federal Food, Drug, and Cosmetic Act (FFDCA), as amended (21 U.S.C. 301 *et seq.*), pesticides added to (or contained in) raw agricultural commodities generally are considered to be unsafe unless a tolerance or exemption from tolerance has been established. Residue tolerances for pesticides are established by EPA under the FFDCA, and the U.S. Food and Drug Administration (FDA) enforce the tolerances set by EPA. Bayer submitted the appropriate regulatory package to EPA for registering the use of glyphosate herbicide on GBH614 cotton. Safe use of glyphosate has been established by the EPA through the registration of glyphosate for use on cotton and the setting of tolerances for the herbicide (EPA 2007).

The FDA policy statement concerning regulation of products derived from new plant varieties, including those genetically engineered, was published in the Federal Register on May 29, 1992, and appears at 57 FR 22984-23005. Under this policy, FDA uses what is termed a consultation process to ensure that human food and animal feed safety issues or other regulatory issues (e.g., labeling) are resolved prior to commercial distribution of bioengineered food. Cotton seed oil is used in the food industry and cotton seed meal is an excellent source of protein and used in animal feed. In compliance with the FDA policy, BCS has submitted a food and feed safety and nutritional assessment summary for GlyTol™ cotton to the FDA. This assessment is pending. As of May 29, 2008, FDA has not announced the completion of Bayer's consultation for GlyTol™ cotton, event GHB614 (See http://www.cfsan.fda.gov/~lrd/biocon.html)

D. Highly Uncertain or Involve Unique or Unknown Risks

The NEPA implementing regulations require consideration of the degree to which the possible effects on the human environment are highly uncertain or involve unique or unknown risk (40 CFR § 1508.27(b)(5)). None of the effects on the human environment identified above are highly controversial, highly uncertain, or involve unique or unknown risks. The effects are similar in kind to (and no worse than) those already observed for currently commercially available and widely grown glyphosate tolerant cotton varieties and to those observed for the use of glyphosate and several other herbicides in agriculture production systems.

E. Threatened and Endangered Species

In addition to the analysis of potential impact to non-target organisms described above (Section II, A, 4), APHIS also considered potential impact on TES. In this analysis, APHIS considered the biology of the glyphosate-resistant cotton, as well as typical agricultural practices associated with cultivation of cotton. As mentioned previously, GlyTol™ cotton differs from non-transgenic cotton only in the expression of the 2mEPSPS gene that is responsible for glyphosate resistance and which differs only from the native corn *EPSPS* gene by two amino acids. The GlyTol™ cotton plants do not express additional proteins, natural toxicants, pheromones, hormones, etc. that could directly or indirectly result in killing or interfering with the normal growth, development, or behavior of a TES or endangered species or species proposed for listing. The GlyTol™ cotton plant is not sexually compatible with a federally listed TES or a species proposed for listing and therefore, would not be integrated into a threatened and endangered species' genetic material. Finally, cultivation of GlyTol™ cotton is not expected to differ from typical cotton cultivation. Cotton does not typically grow in unmanaged habitat and would not be expected to invade and/or persist in the natural environment. GlyTol™ cotton is being presented as an additional consumer choice to the market that has already been saturated with Roundup Ready® (glyphosate tolerant) cotton for the past 11 years (USDA-NASS 2007). Therefore, no additional acreage of glyphosate tolerant cotton is expected due to the deregulation of this product. GlyTol™ cotton is not expected to be grown in any new type of habitat which would include those inhabited by TES species.

As required by Section 7 of the Endangered Species Act, APHIS has analyzed the best available data (current scientific literature, historical data, data supplied in the petition by BCS and information from the FWS TES website) and has reached a determination that granting a petition to deregulate glyphosate-resistant cotton (application #06-332-01p) will have "no effect" on federally listed threatened and endangered species or designated critical habitat or habitat proposed for designation. The data on mammalian toxicity allows APHIS to reach a "no effect" determination for the 358 mammals on the TES list plus the proposed mammals for the TES list. Based on this analysis, there is no apparent potential for impact on non-target organisms, including beneficial organisms and no effect is expected on listed TES, species proposed for listing, or their proposed or designated critical habitat, if APHIS were to grant the petition for non-regulated status in whole.

In addition to APHIS' analysis of the data supplied by the applicant, the EPA has concluded that when used according to the label, the pesticide glyphosate does not have unreasonable adverse effects to human health or the environment. To make such determinations, EPA requires more than 100 different scientific studies and tests from applicants (http://www.epa.gov/pesticides/regulating/). Many plant and wildlife species can be found near or in cities, agricultural fields, and recreational areas. Before allowing a pesticide product to be sold on the market, EPA ensures that the pesticide will not pose any unreasonable risks to wildlife and the environment. EPA does this by evaluating data submitted in support of registration regarding the potential hazard that a pesticide may pose to non-target fish and wildlife species. In considering whether to register a pesticide, EPA conducts ecological risk assessments to determine what risks are posed by a pesticide and whether changes to the use or proposed use are necessary to protect the environment. A pesticide cannot be legally used if it has not been registered with EPA's Office of Pesticide Programs.

Based on the continued use of glyphosate-tolerant cotton for the past 11 years and the data presented by the developer for GlyTol™ cotton as well as a thorough scientific literature search, APHIS believes there would be no impact on non-target organisms or Federally-listed threatened or endangered species if this product is deregulated.

F. Other Considerations

1. Biodiversity

Analysis of available information indicates that BCS' glyphosate-tolerant GE cotton does not exhibit traits that would cause increased weediness; nor should it lead to increased weediness of other cultivated cotton or other sexually compatible relatives. Furthermore, it is unlikely to harm non-target organisms common to the agricultural ecosystem or threatened or endangered species recognized by the U.S. Fish and Wildlife Service because of the information known about the EPSPS protein and its history of safe use for over a decade. There has been no intentional genetic change in these plants to affect their susceptibility to disease or insect damage. The glyphosate-tolerant gene is not expected to change any plant pest characteristics. There is no reason to believe that weediness or plant pest characteristics are different between the genetically engineered and non-engineered plants.

APHIS has concluded that gene transfer to wild cotton species in the United States is limited because of ploidy differences (Table 3, page 18-19 of petition or Table 4, this EA), a lack of documented natural out-crossing, and the limited success of interspecific hybrids produced through controlled breeding (Niles and Feaster 1984; Jenkins 1993; Kareiva, Morris et al. 1994). Based on this reasoning, there is no apparent potential for impact to biodiversity if APHIS were to grant the petition for non-regulated status. The biodiversity of cotton germplasm (seed breeding material and seed varieties) would only be slightly enhanced by the addition of a different transformation event for glyphosate tolerant cotton should the petition for non-regulated status be granted.

2. Raw or Processed Agricultural Commodities

APHIS analysis of data on agronomic performance, disease and insect susceptibility, and compositional profiles of GlyTol™ cotton indicate no differences between this cotton and non-transgenic counterparts that would be expected to cause either a direct or indirect plant pest effect on any raw or processed plant commodity from deregulation of GlyTol™ cotton.

APHIS generally analyzes the transgenic line in comparison to the line or variety from which it was derived and/or to a range of conventional varieties. APHIS analyzes these comparisons to determine if GlyTol™ cotton has any pest characteristics greater than the recipient line or other conventional varieties and to determine if there may be any unintended effects from placing the transgene into GlyTol™ cotton. In the petition (06-332-01p, Section VIII and Appendixes 1-4), different comparisons were presented that ranged from plant growth, lodging, seed moisture content, seed weight, interactions with symbiotic nitrogen-fixing bacteria, response to naturally occurring abiotic stresses, and susceptibility to diseases and insects, and nutritional and anti-nutritional components. None of these comparisons provided any indication of increased pest characteristics or a possibility of an unintended effect that would have a bearing on the health or quality of any raw or processed agricultural commodity. A study comparing the seed composition between conventional soybeans and glyphosate-tolerant soybean found no differences between the two varieties (Padgette, Taylor et al. 1995). These types of studies and the comparator compositional analysis required by FDA and USDA have established that there are no differences between conventional crops and glyphosate-tolerant crops in the 11-year history of use. APHIS is not aware of any additional data that can provide appropriate information for making a proper and reasonable comparison to determine whether GlyTol™ cotton has the potential to impact the human environment.

While FDA is the agency responsible for determining food and feed safety, APHIS analyzed and considered the effects of the action alternatives on food safety as one aspect of public health consistent with APHIS' requirements under NEPA. APHIS reviewed the compositional test results of GlyTol™ cotton in comparison to the non-transformed recipient line and to conventional varieties as presented on p127 of the petition and found no differences between the transformed and non-transformed varieties. Food and feed from GlyTol™ cotton are the subject of a consultation under FDA's consultation procedures for foods derived from new plant varieties (http://www.cfsan.fda.gov). BCS has also applied for approval from Canadian and Mexican markets that would use herbicide-resistant cotton for food, feed or fiber. BCS will be applying for approval in the EU and Japan markets after U.S. approval. Currently, Monsanto's sister product has regulatory food and feed approval in Canada (although not grown in Canada), Australia (planting restricted south of 22°S latitude), Japan, Korea, Mexico, Philippines, and the United States. Since this product is not new or novel and will be marketed as a consumer option, no new impacts from its raw or processed agricultural commodities are expected.

Based on APHIS' analysis, there is no apparent potential for impact to raw or processed agricultural commodities, and therefore there is unlikely to be an impact to public health through direct or indirect consumption of such products, if APHIS were to grant the petition for non-regulated status in whole. If APHIS chooses the no action alternative, there would also be no impact to raw or processed agricultural commodities since most of the present area of cotton production in the United States is already glyphosate tolerant varieties.

3. Current agricultural practices including organic farming

Use of glyphosate-tolerant cotton can provide positive impacts on agricultural practices. These positive impacts have been detailed in a study by Brookes and Barfoot (Brookes and Barfoot 2006) and include:

a) Improved weed control which reduces harvesting costs – cleaner crops have resulted in reduced times for harvesting.

b) Facilitates the use of no-till or reduced-till plowing.

c) Reduced fuel use from less frequent herbicide applications and a reduction in the energy use in soil cultivation.

GE crop technology has provided an additional tool for growers to control competing weeds, reducing the need to rely on soil cultivation and seed-bed preparation as means to getting good levels of weed control. The use of GE crop technology has also reduced the potential damage caused by soil-incorporated residual herbicides in follow-up crops. Under traditional herbicide applications with conventional crops, a post-emergent herbicide application may result in 'knock-back' (crop damage from the residual herbicide application); this problem is less likely to occur in GE herbicide-tolerant crops (Brookes and Barfoot 2006).

The adaptation of no-till or reduced till systems results in time savings and equipment usage. While no- or reduced-till systems are not new, the resultant weed control of GE herbicide-tolerant crops allows the farmer to continue with the no-till/reduced-till systems long after conventional crops necessitate going back to full plowing due to excessive weeds (Brookes and Barfoot 2006).

There are beneficial fuel savings associated with making fewer herbicide applications (relative to conventional crops) and the switch to reduced- and no-till farming systems. Brookes and Barfoot (Brookes and Barfoot 2006) determined that the fuel savings has also resulted in permanent savings in carbon dioxide emissions. In 2005 this amounted to about 2.1 billion pounds (arising from reduced fuel use of 94 million gallons).

> "Over the period 1996 to 2005 the cumulative permanent reduction in fuel use is estimated at 4,613 million kg [10.2 billion lbs] of carbon dioxide (arising from reduced fuel use of 1,679 million litres [443.5 million gallons]; the use of 'no-till' and 'reduced-till' farming systems. As a result, tractor fuel use for tillage is reduced, soil quality is enhanced and levels of soil erosion cut. In turn more carbon remains in the soil and this leads to lower GHG [greenhouse gas] emissions. In 2005, the permanent carbon dioxide savings from reduced fuel use were the equivalent of removing nearly 0.43 million cars from the road; Cumulatively since 1996, the permanent carbon dioxide savings from reduced fuel consumption since the introduction of GM crops are equal to removing 2.05 million cars from the road for one year. In total, the combined GM crop-related carbon dioxide emission savings from reduced fuel use and additional soil carbon sequestration in 2005 were equal to the removal from the roads of nearly 4 million cars" (Brookes and Barfoot 2006)

The National Organic Program administered by USDA's Agricultural Marketing Service requires organic production operations to have distinct, defined boundaries and buffer zones to prevent unintended contact with prohibited substances from adjoining land that is not under organic management. Organic production operations must also develop and maintain an organic production system plan approved by their accredited certifying agent. This plan enables the production operation to achieve and document compliance with the National Organic Standards, including the prohibition on the use of excluded methods. Excluded methods include a variety of methods used to genetically modify organisms or influence their growth and development by means that are not possible under natural conditions or processes. Organic certification involves oversight by an accredited certifying agent of the materials and practices used to produce or handle an organic agricultural product. This oversight includes an annual review of the certified operation's organic system plan and on-site inspections of the certified operation and its records. Although the National Organic Standards prohibit the use of excluded methods, they do not require testing of inputs or products for the presence of excluded methods. The presence of a detectable residue of a product of excluded methods alone does not necessarily constitute a violation of the National Organic Standards. The unintentional presence of the products of excluded methods will not affect the status of an organic product or operation when the operation has not used excluded methods and has taken reasonable steps to avoid contact with the products of excluded methods as detailed in their approved organic system plan. Organic certification of a production or handling operation is a process claim, not a product claim.

It is not likely that farmers, including organic farmers, who choose not to plant transgenic cotton varieties or sell transgenic cotton, will be significantly impacted by the expected commercial use of this product. Non-transgenic cotton will likely still be sold and will be readily available to those who wish to plant it. If BCS receives regulatory approval from all appropriate agencies, it will make the GlyTol™ cotton available to growers or breeders. As with other varieties of cotton, growers or breeders will inquire about the genetic background of this cotton variety and therefore know that this product is a transgenic glyphosate-resistant cotton.

In 2005, of the 13.7 million acres of cotton was grown in the United States (USDA-ERS 2005), 9,537 acres (0.07%) were certified organic cotton (USDA-ERS 2005). USDA-ERS data for 2006 and 2007 were unavailable at this time of writing this EA. The Organic Trade Association (OTC) has the estimates for U.S. certified organic cotton acreage in 2006 as 5,971 acres and 2007 as 7,473 acres (http://www.ota.com/organic/environment/cotton_environment.html). In a 2007 study (Swezey, Goldman et al. 2007), it was estimated that over a 6-year period that cost per production of organic cotton per bale was 37% higher than conventional cotton due to greater hand-weeding costs and lower yields. There was also a lower lint quality due to coloration of the lint in organic cotton. The production prices and lower yields combined with lower prices for cotton were considered the primary obstacles for continued organic production in the study area (Northern San Joaquin Valley, CA).

It is not likely that organic farmers or other farmers who choose not to plant or sell GlyTol™ cotton or other transgenic cotton will be significantly impacted by the expected commercial use of this product as: (a) non-transgenic cotton will likely still be sold and will be readily available to those who wish to plant it; (b) cotton is a highly self-pollinated plant and therefore buffer requirements would be minimal in the absence of pollinators (Van Deynze, Sunderstrom et al. 2005); and (c) 87% of the 2007 cotton acreage in the United States is already planted with transgenic glyphosate-tolerant varieties (Sankula 2006), (d) APHIS expects GlyTol™ cotton to replace some of the presently available glyphosate tolerant cotton varieties without affecting the overall total cotton acreage or glyphosate-tolerant cotton acreage so organic farmers will be able to coexist with biotech cotton producers as they do now. Based on this reasoning, there is no apparent potential for significant impact to organic farming if APHIS were to grant the petition for non-regulated status in whole. If APHIS chooses the no action alternative, there would also be no impact to organic farming since most of the present area of cotton production in the United States already consists of glyphosate tolerant varieties. This particular product should not present new and different issues than those associated with non-transgenic cotton, with respect to impacts on conventional or organic farming. No additional acreage is anticipated to be planted because of the deregulation of this product since glyphosate-resistant cotton is already available on the market and has been available for 11 years.

4. Executive Orders

Executive Order (EO) 12898, "Federal Actions to Address Environmental Justice in Minority Populations and Low-Income Populations," requires Federal agencies to conduct their programs, policies, and activities that substantially affect human health or the environment in a manner so as not to exclude persons and populations from participation in or benefiting from such programs. It also enforces existing statutes to prevent minority and low-income communities from being subjected to disproportionately high and adverse human health or environmental effects.

EO 13045, "Protection of Children from Environmental Health Risks and Safety Risks," acknowledges that children may suffer disproportionately from environmental health and safety risks because of their developmental stage, greater metabolic activity levels, and behavior patterns, as compared to adults. The EO (to the extent permitted by law and consistent with the agency's mission) requires each Federal agency to identify, assess, and address environmental health risks and safety risks that may disproportionately affect children. Each alternative was analyzed with respect to EO 12898 and 13045. None of the alternatives are expected to have a disproportionate adverse effect on minorities, low-income populations, or children.

Each alternative was analyzed with respect to the above **EO 12898** and **13045**. The human health and environmental impacts of the action alternatives are presented in pages 30-32 of the submitted petition. No human health or environmental effects were identified in the data on pages 30-32 of the submitted petition for any of the action alternatives that would have a disproportionate adverse effect or that would exclude a particular group of persons or populations, including minority and low-income populations, or children, from expected benefits. No change is expected in herbicide (or other pesticide applications) or the rate of development of glyphosate-resistant weeds regardless of the alternative chosen. The selection of glyphosate resistance does not disproportionately affect minority and low-income populations or children. Additional analyses provided here indicate that glyphosate resistant cotton technology can provide environmental and economic value to rural agricultural communities. Comparisons of weed management programs for conventional and herbicide resistant cotton were evaluated in a two year (1996-1997) study in across Alabama. Glyphosate resistant cotton was shown to have lower herbicide injury levels and higher weed control levels in a total post-emergence herbicide program, while maintaining high yield and greater net returns. Net returns were determined more by weed control and variety yield potential than by treatment cost. The economic and environmental impacts of glyphosate-resistant crops were reviewed by Gianessi (Gianessi 2005). It was estimated in the year 2000 that use of glyphosate-tolerant cotton has saved the industry approximately $132 million dollars per year by reducing herbicide applications, tillage and hand-weeding (Gianessi 2005). Crop safety is also a concern for the farmer, as well as to their children and pesticide applicators. Of 182 alternative herbicide treatment programs available for use on cotton, glyphosate was among the 47 with the highest crop safety rating in the weed control guides (Gianessi 2005). In another simulation study, researchers have looked at the effect of switching from glyphosate-resistant crops to conventional seeds with other herbicides, and they found that the switch would require farmers to increase the LD_{50} dose applied to the average U.S. farm by 25% per hectare in cotton (Service 2007). The LD_{50} dose is a mammalian toxicity measure for the volume of pesticide needed to kill 50% of a test population of rats. Even with conventional tillage, the use of glyphosate resistant crops reduces the number of LD_{50} doses applied per hectare (Service 2007). Under the "no action" alternative these benefits would presumably continue. If the petition is granted in whole, these benefits would also presumably continue and may be even greater if the varieties developed from GlyTol™ cotton are higher yielding as anticipated by the developer.

EO 13112, “Invasive Species”, states that federal agencies take action to prevent the introduction of invasive species and provide for their control and to minimize the economic, ecological, and human health impacts that invasive species cause. Cotton is not considered an invasive species, is readily out-grown by weeds and does not establish itself without human intervention (as described in on page 21, Appendix I). Based on historical experience with cotton and the agronomic data submitted by the applicant (pages 121-132, petition data) and reviewed by APHIS, the engineered plant is sufficiently similar in fitness characteristics to other cotton varieties currently grown and it is not expected to have an increased invasive potential.

Executive Order 12114, “Environmental Effects Abroad of Major Federal Actions” requires Federal officials to take into consideration any potential environmental effects outside the U.S., its territories and possessions that result from actions being taken. APHIS has given this due consideration and does not expect an environmental impact outside the United States should APHIS choose any of the listed alternatives to petition #06-332-01p. It should be noted that all the considerable, existing national and international regulatory authorities and phytosanitary regimes that currently apply to introductions of new cotton cultivars internationally, apply equally to those covered by an APHIS determination of non-regulated status under 7 CFR part 340. Any international traffic of genetically engineered cotton subsequent to a determination of regulated status for GE cotton would be fully subject to national phytosanitary requirements and be in accordance with phytosanitary standards developed under the International Plant Protection Convention (IPPC).

The purpose of the IPPC “is to secure a common and effective action to prevent the spread and introduction of pests of plants and plant products and to promote appropriate measures for their control” (https://www.ippc.int/IPP/En/default.jsp). The protection it affords extends to natural flora and plant products and includes both direct and indirect damage by pests, including weeds. The IPPC has set a standard for the reciprocal acceptance of phytosanitary certification among the nations that have signed or acceded to the Convention (137 countries as of April 2005). In April, 2004, a standard for pest risk analysis (PRA) of living modified organisms (LMOs) was adopted at a meeting of the governing body of the IPPC as a supplement to an existing standard, International Standard for Phytosanitary Measure No. 11 (ISPM-11; Pest Risk Analysis for Quarantine Pests). The standard acknowledges that all LMOs will not present a pest risk, and that a determination needs to be made early in the PRA for importation as to whether the LMO poses a potential pest risk resulting from the genetic modification. APHIS pest risk assessment procedures for bioengineered organisms are consistent with the guidance developed under the IPPC. In addition, issues that may relate to commercialization and transboundary movement of particular agricultural commodities produced through biotechnology are being addressed in other international forums and through national regulations.

The Cartagena Protocol on Biosafety is a treaty under the United Nations Convention on Biological Diversity (CBD) that established a framework for the safe transboundary movement, with respect to the environment and biodiversity, of LMOs, which includes those modified through biotechnology. The Protocol came into force on September 11, 2003 and 132 countries are Parties to it as of March 6, 2006 (see http://www.biodiv.org/biosafety/default.aspx). Although the United States is not a party to the CBD, and thus not a party to the Cartagena Protocol on Biosafety, US exporters will still need to comply with domestic regulations that importing countries that are Parties to the Protocol have put in place to comply with their obligations. The first intentional transboundary movement of LMOs intended for environmental release (field trials or commercial planting) will require consent from the importing country under an advanced informed agreement (AIA) provision, which includes a requirement for a risk assessment consistent with Annex III of the Protocol, and the required documentation. LMOs imported for food, feed or processing (FFP) are exempt from the AIA procedure, and are covered under Article 11 and Annex II of the Protocol. Under Article 11 Parties must post decisions to the Biosafety Clearinghouse database on domestic use of LMOs for FFP that may be subject to transboundary movement. To facilitate compliance with obligations to this protocol, the US Government has developed a website that provides the status of all regulatory reviews completed for different uses of bioengineered products (http://usbiotechreg.nbii.gov). This data will be available to the Biosafety Clearinghouse.

APHIS continues to work toward harmonization of biosafety and biotechnology consensus documents, guidelines and regulations, including within the North American Plant Protection Organization (NAPPO), which includes Mexico, Canada, and the United States and in the Organization for Economic Cooperation and Development. NAPPO has completed three modules of a standard for the *Importation and Release into the Environment of Transgenic Plants in NAPPO Member Countries* (see http://www.nappo.org/Standards/Std-e.html). APHIS also participates in the North American Biotechnology Initiative (NABI), a forum for information exchange and cooperation on agricultural biotechnology issues for the U.S., Mexico and Canada. In addition, bilateral discussions on biotechnology regulatory issues are held regularly with other countries including: Argentina, Brazil, Japan, China, and Korea.

EO 13175, “Consultation and Coordination with Indian Tribal Governments”, requires regular and meaningful consultation and collaboration with tribal officials in the development of Federal policies that have tribal implications. USDA is the lead agency of the Federal Government for providing effective and efficient coordination of Federal agricultural and rural development programs. Consistent with applicable law, USDA officials consult with tribal governments and Alaskan Native Corporations (ANC) regarding the influence of USDA activities on water, land, forest, air, and other natural resources of tribal governments and ANCs. USDA-APHIS responded to EO 13175 by establishing the APHIS Native American Working Group (ANAWG), which has representatives from all APHIS program areas. The group advises the agency's top management about ways to enhance program delivery and accessibility to tribes, intertribal committees, and related organizations, such as the Intertribal Agriculture Council. APHIS, BRS has an active representative in the ANAWG and works in partnership with both Indian Tribal Governments and the APHIS Management Team (AMT) during permitting and deregulation of plants that can affect any tribal areas. The deregulation of GlyTol™ cotton does not have any tribal land impacts as it is to be marketed as a consumer option to a product that has already existed in the market for the past 11 years.

EO 13186, “Responsibilities of Federal Agencies to Protect Migratory Birds” requires an agency to have a Memorandum of Understanding (MOU) with the Fish and Wildlife Service (FWS) that shall promote the conservation of migratory bird populations. The Migratory Bird Treaty Act of 1918 as amended and Executive Order 13186 states that migratory birds include all native wild birds found in the United States except the house sparrow, starling, feral pigeon, and resident game birds such as pheasant, grouse, quail, and wild turkeys. A reference list of migratory game birds is found in Title 50, CFR, part 10. The Migratory Bird Treaty Act makes it unlawful for anyone to kill, capture, collect, possess, buy, sell, trade, ship, import, or export any migratory bird, including feathers, parts, nests, or eggs. Executive Order 13186 “Responsibilities of

Federal Agencies to Protect Migratory Birds" requires Federal officials to consider the impacts of planned actions on migratory bird populations and habitats for all planning activities. APHIS has determined that it is reasonable to assume that the deregulation of GlyTol™ cotton should have no impact on migratory birds since glyphosate tolerant cotton already exists in the marketplace and no adverse effects have been noted on any bird species within the Cotton Belt.

V. Listing of Agencies and Persons Consulted

Biotechnology Regulatory Services
Mike Gregoire, Deputy Administrator

Permits and Risk Assessment Staff
Neil Hoffman, Ph.D., Division Director (Reviewer)
Aimee Hyten, PhD. Biotechnologist (Reviewer)
Michael Watson, PhD. Plants Branch Chief (Reviewer)

BRS, Policy and Coordination Division
John Turner, Ph.D., Director
Patricia Beetham, Ph.D. Biotechnologist (EA Preparer)

Environmental Services
Rhonda Solomon, Ph.D. Reviewer

VI. References

Brookes, G. and P. Barfoot (2006). "Global impact of biotech crops: Socio-economic and environmental effects in the first ten years of commercial use." AgBioForum **9**: 139-151.

Brown, J. R. (2003). "Ancient horizontal gene transfer." Genetics **4**: 121-132.

Burgos, N., S. Culpepper, et al. (2006). "Managing Herbicide Resistance in Cotton Cropping Systems."

Codex (2003). Codex principles and guidelines on food derived from biotechnology. Rome, Codex Alimentarius Commission**:** 37.

Crockett, L. (1977). Wildly Successful Plants: North American Weeds. Honolulu, HI, University of Hawaii Press.

Crockett, L. (1977). Wildly successful plants: North American weeds. Honolulu, Hawaii, University of Hawaii.

EFSA (2007). EFSA statement on the fate of recombinant DNA or proteins in meat, milk and eggs from animals fed with GM feed, European Food Safety Authority.

Endrizzi, J. E., E. I. Turcotte, et al. (1984). Cotton. Agronomy No. 24. R. J. Kohel and C. F. Lewis. Wisconsin, USA, Soil Science Society of America, Inc.**:** 82-129.

EPA (2007). "Glyphosate; Pesticide tolerances. 40 CFR part 180.364."

Fryxell, P. A. (1979). The natural history of the cotton tribe (*Malvaceae*, tribe *Gossypieae*). College Station and London, Texas A&M University Press.

Gebhard, F. and K. Smalla (1999). "Monitoring field releases of genetically modified sugar beets for persistence of transgenic plant DNA and horizontal gene transfer." FEMS Microbiology Ecology **28**: 261-272.

Gianessi, L. P. (2005). "Economic and herbicide use impacts of glyphosate-resistant crops." Pest Management Science **61**(3): 241-245.
Holm, L., J. V. Pancho, et al. (1979). A Geographical Atlas of World Weeds. New York, NY, John Wiley and Sons.
Holm, L., J. V. Pancho, et al. (1979). A geographical atlas of world weeds. NY, John Wiley and Sons.
Humacher, R. B. and S. D. Wright. (2006). "Methods to enable the coexistence of diverse cotton production systems, Publication 8191." Agricultural Biotechnology in California Series.
IPCS (1994). Glyphosate. Environmental Health Criteria 159. P. Janssen and H. Mensink. Geneva, World Health Organization.
Jenkins, J. N. (1993). Cotton. Traditional crop breeding practices: a historical review to serve as a baseline for assessing the role of modern technology., OECD.
Kaneko, T., Y. Nakamura, et al. (2000). "Complete genome structure of the nitrogen-fixing symbiotic bacterium *Mesorhizobium loti*." DNA Research **7**(6): 331-338.
Kareiva, P., W. Morris, et al. (1994). "Studying and managing the risk of cross-fertilization between transgenic crops and their wild relatives." Molecular Ecology **3**: 15-21.
Kishore, G. M. and D. M. Shah (1988). "Amino acid biosynthesis inhibitors as herbicides." Annu Rev Biochem **57**: 627-63.
Koonin, E. V., K. S. Makarova, et al. (2001). "Horizontal gene transfer in prokaryotes: Quantification and classification." Annual Review of Microbiology **55**: 709-742.
Malik, J., G. Barry, et al. (1989). "The herbicide glyphosate." Biofactors **2**(1): 17-25.
McGregor, S. E. (1976). Insect pollination of cultivated crop plants. Agricultural Handbook No 496. Washington, DC, USDA-ARS.
Muenscher, W. C. (1980). Weeds, 2nd Edition. Ithaca and London, Cornell University Press.
Muenscher, W. C. (1980). Weeds. Second Edition. New York and London, Cornell University Press.
Niles, G. A. and C. V. Feaster (1984). Cotton. Agronomy No. 24. R. j. Kohel and C. F. Lewis. Wisconsin, USA, Soil Science of America, Inc.**:** 205.
OECD (2004). "Consensus Document on compositional considerations for new varieties of cotton (*Gossypium hirsutum* and *Gossypium barbadense*): key food and feed nutrients and anti-nutrients." **ENV/JM/MONO(2004)16**.
Padgette, S. R., N. B. Taylor, et al. (1995). "The composition of glyphosate-tolerant soybean seeds is equivalent to that of conventional soybeans." Journal of Nutrition **126**: 702-716.
Sankula, S. (2006). Quantification of the impacts on US agriculture of biotechnology-derived crops planted in 2005., National Center for Food and Agricultural Policy**:** 12.
Sankula, S., G. Marmon, et al. (2005). Biotechnology-derived crops planted in 2004-Impacts on US Agriculture. Washington, DC, National Center for Food and Agricultural Policy.
Schlüter, K., J. Fütterer, et al. (1995). "Horizontal gene transfer from a transgenic potato line to a bacterial pathogen (*Erwinia chrysanthemi*) occurs if at all at an extremely low frequency." Biotechnology **13**: 1094-1098.
Service, R. F. (2007). "Glyphosate - The conservationist's friend?" Science **316**: 1116-1117.
Steinrucken, H. C. and N. Amrhein (1980). "The herbicide glyphosate is a potent inhibitor of 5-enolpyruvyl-shikimic acid-3-phosphate synthase." Biochem Biophys Res Commun **94**(4): 1207-12.
Swezey, S., P. Goldman, et al. (2007). "Six-year comparison between organic, IPM and conventional cotton production systems in the Northern San Joaquin Valley, California." Renewable Agriculture and Food Systems **22**(1): 30-40.

USDA-APHIS. (2006). "Plant Health: Noxious Weeds Program." Retrieved 08/22/2007, from http://www.aphis.usda.gov/plant_health/plant_pest_info/weeds/index.shtml.
USDA-ERS. (2005). "Cotton and Wool Outlook." Retrieved 08/23/2007, from http://usda.mannlib.cornell.edu/usda/ers/CWS//2000s/2005/CWS-12-13-2005_revision.pdf.
USDA-ERS. (2005). "Organic Production." Retrieved 08/23/2007, from http://www.ers.usda.gov/data/organic/.
USDA-ERS. (2006). "The First Decade of Genetically Engineered Crops in the United States. Economic Information Bulletin No. 11."
USDA-ERS. (2006). "The First Decade of Genetically Engineered Crops in the United States. Economic Information Bulletin No. 11." Retrieved 08/23/2006, from http://www.ers.usda.gov/publications/eib11/eib11.pdf.
USDA-FAS (2006). Cotton: World market events and trade. Circular Series FOP 9-06. USDA-FAS**:** 26.
USDA-NASS (2007). Acreage, National Agricultural Statistics Service (NASS).
Van Deynze, A. E., F. J. Sunderstrom, et al. (2005). "Pollen-mediated gene flow in California cotton depends on pollinator activity." Crop Science **45**: 1565-1570.

VII. SELECTED RESOURCE MATERIALS

USDA-APHIS documents:
Petition submitted by the developer

Environmental Protection Agency documents:
June 2003. EPA's Regulation of Biotechnology for Use in Pest Management. (http://www.epa.gov/pesticides/biopesticides/reg_of_biotech/eparegofbiotech.htm)

Food and Drug Administration documents:
March 2007. CFSAN/Office of Food Additive Safety. List of Completed Consultations on Bioengineered Foods (http://www.cfsan.fda.gov/~lrd/biocon.html)

OECD 1999. OECD Environmental Health and Safety Publications. Series on Harmonization of Regulatory Oversight in Biotechnology, No. 10. Consensus Document on General Information Concerning the Genes and Their Enzymes that Confer Tolerance to Glyphosate Herbicide (http://www.oecd.org/ehs/).

OECD 2004. Environment Directorate Joint Meeting of the Chemicals Committee and The Working Party on Chemicals, Pesticides and Biotechnology. Series on the Safety of Novel Foods and Feeds, No. 11. Consensus Document on Compositional Considerations for New Varieties of Cotton (*Gossypium hirsutum* and *Gossypium barbadense*): Key Food and Feed Nutrients and Anti-Nutrients (http://www.oecd.org/ehs/).

USDA-ERS Organic Handler Database information:
from http://www.ers.usda.gov/Data/OrganicHandlers/ :
"The ERS organic handler database contains select results from the 2004 Nationwide Survey of Organic Manufacturers, Processors, and Distributors, administered by Washington State University, Social and Economic Sciences Research Center. The survey covered a variety of

topics related to the procurement and contracting of organic products and ingredients. Data are available on 9 commodity groups, such as fruit and nuts, and 45 commodities, such as berries and citrus. The procurement data include information from 1,038 facilities; the contracts data include information from 686 facilities that use contracts."

Appendix A: Pest Risk Assessment

In evaluating plant pest risk, APHIS reviews the petition along with scientific evidence to determine if there are differences between GlyTol™ cotton and traditional Upland cotton with regard to plant pest risk. APHIS regulations 7 CFR part 340 defines a plant pest as:

> "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"

Plant pest risk evaluation by APHIS includes comparing the GE plant and unmodified plant to determine if the inserted gene affects weediness, the impact on the weediness of another other plant with which it can interbreed, transfer of genetic information to organisms with which it cannot interbreed and differences in disease and pest susceptibilities. APHIS made these evaluations by comparing monitored agronomic properties in prior field tests done by the applicant. Field test reports can be found in the BCS GlyTol™ cotton petition in Appendix 1 (petition# 06-332-01p, p.86-105). Agronomic, gene expression and protein characterization data from the field tests can be found in the submitted petition (petition# 06-332-01p, p.108-173).

A. Weeds and Resistance

1. Upland Cotton

In the United States, Upland cotton (*Gossypium hirsutum*) is not a weed pest (Crockett 1977; Holm, Pancho et al. 1979; Muenscher 1980). Upland cotton is a domesticated crop that requires human intervention to survive in non-cotton production areas.

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 lists of noxious weed species 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 (see Table 2 for pre-emergent herbicides) or mechanical means. It does not compete effectively with cultivated plants or primary colonizers (OECD 2004).

2. Glyphosate resistant cotton

The addition of herbicide tolerance in BCS' GE cotton does not confer any additional weediness potential. Eleven years experience with Roundup Ready® cotton has demonstrated that it is an additional tool in integrated weed management systems (http://www.weedresistancemanagement.com/layout/default.asp). Glyphosate-tolerant plants are susceptible to many other herbicides other than glyphosate (see Table 1). Volunteer plants can easily be controlled by pre- or post-emergence herbicides as indicated in the

University of California’s website on integrated weed management (http://www.ipm.ucdavis.edu/PMG/r114700111.html).

APHIS believes there will be no plant pest risk impacts due to increased weediness from this GE cotton based on the absence of such weediness observed during the prior commercial use of herbicide-tolerant cotton grown on an accumulated 78.5 million acres over the last 11 years (data compiled from NASS and ERS data sets; http://www.nass.usda.gov and http://www.ers.usda.gov/Data/biotechcrops/). Because glyphosate tolerant cotton has already been highly adopted by U.S. cotton growers, this new product is not expected to lead to an increase in the US acreage of glyphosate tolerant cotton. APHIS believes the deregulation of this product will not cause an increase in the US acreage of glyphosate tolerant cotton, but simply provide an additional consumer choice. APHIS also believes that there is no apparent potential for significant impact to plant pest risk from stacking of herbicide resistance traits if APHIS were to grant the petition for non-regulated status.

The potential impacts of stacking of herbicide resistance traits (e.g. combining two or more traits through crossing of different genetically engineered plants) are the availability of deregulated herbicide resistance events, the effect of stacked traits on the plant and on herbicide use, the number of effective alternative herbicides for cotton production, the probability of developing weeds with multiple resistance to various herbicide modes of action and the probability of a stacked cotton becoming a weed. APHIS has previously deregulated other herbicide tolerance gene/events in cotton. The first herbicide tolerant cotton to be deregulated was the glyphosate-tolerance cotton based on the *cp4 EPSPS* gene by Monsanto in 1995. The second herbicide tolerance trait to be deregulated in cotton was tolerance to the phosphinothricin class of herbicides based on expression of phosphinothricin-N-acetyl transferase (PAT) enzyme, which catalyzes the conversion of the active herbicidal ingredient glufosinate ammonium to an inactive form. There are two types of genes that encode similar PAT enzymes; i.e. the *bar* gene from *Streptomyces hygroscopicus* and a synthetic *pat* gene derived from *Streptomyces viridochromogenes.*

Based on all of the genetically engineered herbicide tolerant traits in all of the crops deregulated to-date by APHIS, herbicide tolerant traits that have been deregulated for cotton have no effect on any other plant characteristic (see agronomic data petition# 06-332-01p, p.105-173) so the stacking of two or more herbicide tolerant traits into one plant should have no effect on making the plant more weedy or changing the level of other herbicide tolerances in the plant. As noted above in Section II (Affected Environment), several alternative herbicides are necessary to use in cotton for controlling a wide array of weeds. The development of herbicide resistant weeds is generally due to frequent use of the same herbicide over a period of time on the same area. Alternating herbicides with different modes of actions to control weeds generally is recommended to help avoid the development of herbicide resistant weeds, and successful cotton producers incorporate this into their agricultural and cultivation practices. Therefore incorporating tolerance to two or more herbicides into the same plant may be useful in avoiding the development of herbicide resistant weeds. Cotton has never been considered a weed other than as an occasional volunteer in subsequent crops.

B. Gene introgression[6] from GE cotton into its sexually compatible relatives

Potential impacts to be addressed are those that pertain to the use of GlyTol™ cotton and its progeny in the absence of confinement. Does the presence of the 2mEPSPS protein in GlyTol™ cotton confer any advantage over the unmodified cotton plant?

In assessing the risk of gene introgression from BCS' glyphosate-resistant 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 transfer[7]; and 2) the potential plant pest risk of introgression.

1. 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*. *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 (like only pollinating at night as in the case of *G. tomentosum*) or biological impediments (geography or absence of pollinators) reduce the potential for pollen movement (Fryxell 1979). Table 4 outlines the compatibility of all species on an international level.

Table 4. Cotton Species

Species	Common Name	Native location	Comments
G. hirsutum	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 *G. barbadense* and *G. tomentosum*.
G. barbadense	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 *G. hirsutum* and *G. tomentosum*.
G. tomentosum	Ma'o or Hawaiian cotton	Hawaii	Non-commercial species. Pollinated by moths when the flowers open at night. Only found in Hawaii. Sexually compatible with *G. hirsutum* and *G.*

[6] Introgression is the introduction of genes from one species into the gene pool of another via sexual crossing. The process begins with hybridization between the two species, followed by repeated backcrossing to one of the parent species.
[7] Horizontal gene transfer is any process in which an organism transfers genetic material to another cell that is not its offspring.

			barbadense.
G. arboreum	Asiatic tree or tree cotton	Pakistan, India	Commercial species, grown in Europe, Africa and eastern countries. Sexually compatible with *G. herbaceum*.
G. herbaceum	Levant cotton	Africa, Arabia	Commercial species, grown in Europe, Africa and eastern countries. Sexually compatible with *G. arboreum*.
G. thurberi	Thurber's, Desert or Arizona desert cotton	Mexico, Arizona	Non-commercial species. Sexually compatible with *G. arboreum* and *G. herbaceum*.

Cross-pollination between *G. tomentosum* and *G. hirsutum* is unlikely because they use different insect pollinators and are receptive to pollination at different times of the day. Flowers of *G. tomentosum* are pollinated by moths at night unlike flowers of *G. hirsutum* which are pollinated by bees during the day (MacGregor 1976). Concentration of suitable pollinators varies from location to location and by season, and is considerably suppressed by insecticide use.

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"[8]. Since GlyTol™ 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.

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 (Humacher and Wright 2006).

[8] From AOSCA "Yellow Books" 2003 OPERATIONAL PROCEDURES, CROP STANDARDS AND SERVICE PROGRAMS PUBLICATION (Genetic and Crop Standards), pg 194.

2. Gene Flow via Horizontal Gene Transfer[9]

Transfer and expression of DNA from GlyTol™ 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.

3. Summary

APHIS believes that natural gene transfer in cotton is such an unlikely event that there is minimal risk for gene introgression via gene transfer. If gene introgression were to occur via pollen flow, cotton is not considered a weed and the gene event would not confer any additional survival advantage over non-GE cotton. There would also be no impact from introgression since 87% of the present area of cotton production in the United States is already planted with herbicide tolerant varieties (herbicide-tolerant or herbicide-tolerant stacked with insect-resistance) (USDA-NASS 2007).

---

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

Appendix B: Technical Information about Glyphosate and *EPSPS* Gene

A. Glyphosate

The glyphosate herbicide (N-phosphonomethyl-glycine) is registered for non-selective weed control on both non-food use and food use plants. Glyphosate is a systemic herbicide that has a relatively slow mode of action allowing for the movement of the herbicide throughout the plant before symptoms occur. It has been found to be biodegradable and acute toxicity studies have demonstrated the lack of toxic effects in humans and wild fauna (Malik, Barry et al. 1989).

Glyphosate works by interfering with normal plant metabolism by competing with the naturally present enzyme, 5-enolpyruvyl-3-phosphoshikimate acid synthase (EPSPS). EPSPS is involved in the biosynthesis of the aromatic amino acids, phenylalanine, tryptophan, and tyrosine (as well as some secondary metabolites) through the shikimate pathway. These aromatic amino acids are essential building blocks of proteins in all species. The herbicide glyphosate resembles the structure of the substrate for EPSPS, phosphoenolpyruvate (PEP). Therefore, glyphosate competes with PEP for the enzyme's active site and prevents the conversation of PEP to the molecule that is required for the synthesis of aromatic amino acids. As a consequence of interfering with aromatic amino acid biosynthesis, plant cells cannot complete the synthesis of proteins and the plant dies (Kishore and Shah 1988). EPSPS is found naturally in all plants, fungi and some bacteria but is not present in animals (including humans). For animals, aromatic amino acids must be obtained through the diet (Steinrucken and Amrhein 1980). Consequently, all animals are naturally exposed to sources of EPSPS through their normal diets.

B. Use of an *EPSPS* gene

To create a plant that is resistant to glyphosate herbicide, the EPSPS enzyme must be mutated, but not inactivated. It still must be able to function in the shikimate pathway to produce essential amino acids, but not be able to bind glyphosate herbicide. The first mutated EPSPS enzyme that was placed into corn came from the *Agrobacterium* C4 gene. This gene was identical to the naturally occurring EPSPS protein in corn with the exception of two amino acid mutations. These two amino acid mutations allowed the corn plant to continue to make aromatic amino acids, but did not readily bind to the glyphosate herbicide, allowing the plant's survival in the presence of the herbicide.

The *EPSPS* gene inserted in GlyTol™ cotton is from corn (*Zea mays*) and its protein differs from the native protein by only two amino acids (Table 8, p.40 of submitted petition). BCS has conducted a safety evaluation of the 2mEPSPS protein produced in GlyTol™ cotton including homology searches for allergenicity and toxins, as well as *in vitro* digestibility assays. In keeping with historical data (demonstrated with both *Agrobacterium* EPSPS and corn event GA21 (mEPSPS), BCS' data demonstrates the 2mEPSPS protein is not resistant to *in vitro* digestion and shows no homology with allergens or toxins. Expression data for the protein during plant growth and the verification of biochemical properties and function can be found in BCS' submitted petition on pages 30-40.