Bayer CropScience Petition (17-138-01p) for Determination of Non-regulated Status of Glyphosate and Isoxaflutole Resistant GHB811 Cotton

OECD Unique Identifier:
BCS-GH811-4

Preliminary Plant Pest Risk Assessment

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A. Introduction

Bayer CropScience, LP (hereafter referred to as Bayer) has petitioned the Animal and Plant Health Inspection Service (APHIS) of the United States Department Agriculture (USDA) for a determination that the genetically engineered (GE**)** glyphosate and isoxaflutole (IFT) herbicide-resistant[1] cotton event GHB811 (OECD unique Identifier BCS-GHB811-4) is unlikely to pose a plant pest risk and, therefore, should no longer be a regulated article under the APHIS' 7 Code of Federal Regulations (CFR) part 340. This petition was assigned the number 17-138-01p, and is hereafter referenced as Bayer 2017a. APHIS administers 7 CFR part 340 under the authority of the plant pest provisions of the Plant Protection Act (PPA) of 2000 (7 U.S.C. 7701 *et seq*.)[2]. This plant pest risk assessment was conducted to determine if GHB811 cotton is unlikely to pose a plant pest risk.

APHIS regulations in 7 CFR part 340 regulate the introduction (importation, interstate movement, or release into the environment) of certain GE organisms and products. A GE organism is no longer subject to the plant pest provisions of the PPA or to the regulatory requirements of 7 CFR part 340 when APHIS determines that it is unlikely to pose a plant pest risk. A GE organism is considered a regulated article under 7 CFR part 340 if the donor organism, recipient organism, or vector, or vector agent used in engineering the organism belongs to any genera or taxa designated in 7 CFR § 340.2 and meets the definition of plant pest, or is an unclassified organism and/or an organism whose classification is unknown, or any product which contains such an organism, or any other organism or product altered or produced through genetic engineering which the Administrator determines is a plant pest or has reason to believe is a plant pest[3]. GHB811 cotton was produced by an *Agrobacterium*-mediated method of transformation and portions of the introduced genetic sequences come from plant pest organisms listed in 7 CFR § 340.2 (i.e., promoter sequence from Cassava Vein Mosaic Virus, T-DNA border sequences are from *Agrobacterium tumefaciens*). Therefore, the GE Crop Event GHB811 is considered a regulated article under APHIS regulations at 7 CFR part 340. Bayer has conducted introductions of GHB811 cotton as a regulated article under APHIS-authorized

[1] Bayer has described the phenotype of GHB811 cotton as "herbicide tolerant" and historically APHIS has also referred to GE plants with reduced herbicide sensitivity as herbicide tolerant. However, the phenotype would fall under the Weed Science Society of America (WSSA) definition of "herbicide resistance" since GHB811 cotton has an "inherited ability to survive and reproduce following exposure to a dose of herbicide normally lethal to the wild type". By the WSSA. 1998. *"Herbicide Resistance" and "Herbicide Tolerance" defined. (Technology Note)*. Weed Technology 12, pp. 789. Retrieved from http://www.jstor.org/stable/3989101 Last accessed 04/09/2013. Definition, "resistance (to an herbicide) may be naturally occurring or induced by such techniques as genetic engineering or selection of variants produced by tissue culture or mutagenesis." Herbicide tolerance, by the WSSA definition, only applies to plant species with an "inherent ability to survive and reproduce after herbicide treatment. This implies that there was no selection or genetic manipulation to make the plant tolerant; it is naturally tolerant."

[2] Plant Protection Act in 7 U.S.C. 7702 § 403(14) defines plant pest as: "Plant Pest - The term "plant pest" means any living stage of any of the following that can directly or indirectly injure, cause damage to, or cause disease in any plant or plant product: (A) A protozoan. (B) A nonhuman animal. (C) A parasitic plant. (D) A bacterium. (E) A fungus. (F) A virus or viroid. (G) An infectious agent or other pathogen. (H) Any article similar to or allied with any of the articles specified in the preceding subparagraphs."

[3] Limited exclusions or exemptions apply for certain engineered microorganisms and for interstate movement of some organisms, as in 7 CFR § 340.1 and § 340.2.(b).

notifications since 2012 (Table 8.1, p.120; Bayer 2017a), in part, to gather information to support that GHB811 cotton is unlikely to pose a plant pest risk.

Potential impacts addressed in this Plant Pest Risk Assessment are those that pertain to plant pest risk associated with GHB811 cotton and its progeny and their use in the absence of confinement relative to the unmodified recipient and/or other appropriate comparators. APHIS utilizes data and information submitted by the applicant, in addition to current literature, to determine if GHB811 cotton is unlikely to pose a plant pest risk. APHIS regulations in 7 CFR § 340.6(c) specify the information needed for consideration in a petition for nonregulated status. APHIS will assess information submitted by the applicant about GHB811 cotton related: to plant pest risk characteristics; expression of the gene product, new enzymes, or changes to plant metabolism; disease and pest susceptibilities and indirect plant pest effects on other agricultural products; effects of the regulated article on nontarget organisms; weediness of the regulated article; impact on the weediness of any other plant with which it can interbreed; changes to agricultural or cultivation practices that may impact diseases and pests of plants; and transfer of genetic information to organisms with which it cannot interbreed.

APHIS may also consider information relevant to reviews conducted by other agencies that are part of the 'Coordinated Framework for the Regulation of Biotechnology'(51 FR 23302 1986; 57 FR 22984 1992). Under the Coordinated Framework, the oversight of biotechnology-derived plants rests with APHIS, the Food and Drug Administration (FDA), and the Office of Pesticide Programs of the U.S. Environmental Protection Agency (EPA). Depending on their characteristics, certain biotechnology-derived products are subjected to review by one or more of these agencies.

EPA regulates under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) (7 U.S.C. 136 *et seq)* the distribution, sale, use and testing of pesticidal substances produced in plants and microbes, including those pesticides that are produced by an organism through techniques of modern biotechnology. EPA also sets tolerance limits for residues of pesticides on and in food and animal feed, or establishes an exemption from the requirement for a tolerance, under the Federal Food, Drug and Cosmetic Act (FFDCA; 21 U.S.C. Chapter 9). Prior to registration for a new use for a new or previously registered pesticide, EPA must determine through testing that the pesticide does not cause unreasonable adverse effects on humans, the environment, and nontarget species when used in accordance with label instructions. EPA must also approve the language used on the pesticide label in accordance with 40 CFR part 156. Other applicable EPA regulations include 40 CFR part 152 - Pesticide Registration and Classification Procedures, part 158 – Data Requirements for Pesticides, part 174 - Procedures and Requirements for Plant Incorporated Protectants (PIPs), and part 172 - Experimental Use Permits. A label expansion to allow the use of isoxaflutole on Event GHB811 cotton has been submitted to EPA.

The FDA under the FFDCA is responsible for ensuring the safety and proper labeling of all plant-derived foods and feeds, including those developed through modern biotechnology. To help sponsors of foods and feeds derived from genetically engineered crops comply with their obligations, the FDA encourages them to participate in its voluntary early food safety evaluation for new non-pesticidal proteins produced by new plant varieties intended to be used as food (FDA 2006) and a more comprehensive voluntary consultation process prior to commercial

distribution of food or feed (57 FR 22984 1992). Bayer (2017a) submitted a Premarket Biotechnology Notification (PBN) to FDA on April 17, 2017. To date, an administrative number has not been assigned to this PBN.

B. Development of GHB811 Cotton

Cotton belongs to the genus *Gossypium*, which consists of 49 species, four of which are cultivated in tropical and subtropical regions around the world: *Gossypium hirsutum*, *G. barbadense*, *G. arboretum* and *G. herbaceum* (Fryxell 1979; Fryxell 1984; Wendel and Cronn 2003; OECD 2008; Wendel et al. 2010). *G. hirsutum* (Upland cotton) is the subject of this risk assessment and in 2017 comprised 98% of all cotton grown in the U.S. (USDA-NASS 2017a). *G. barbadense* (Pima or Egyptian cotton) is primarily harvested in California (USDA-NASS 2012) and in 2017 comprised 2% of all cotton grown in the U.S. (OECD 2008; USDA-NASS 2017a). Approximately 96% of all Upland cotton grown in the U.S. has GE modifications: 11% has herbicide-resistant only traits, 5% has arthropod-resistant only traits (usually labelled BT because the source of the traits are from *Bacillus thuringiensis*) and 80% has both traits (USDA-ERS 2017b). The other two cultivated species, *G. arboretum* and *G. herbaceum*, are not grown in the United States (Wendel et al. 2010). Cotton is a perennial cultivated as an annual, and is more limited geographically than other major crops in the United States because it can be grown only in regions with more than 180-200 frost-free days per year (Fryxell 1979; OECD 2008). Cotton has been grown in 17 states from Virginia southward and westward to California in an area often referred to as the Cotton Belt (Figure 1).

Cotton is primarily used for its textile fibers that grow on the seed within the cotton boll (Rost 1998). Cotton is the single most important textile fiber in the world, accounting for about 35% of all fibers produced (Agricultural Marketing Resource Center 2012). Other value-added products from cotton are cottonseed oil and cottonseed meal. Cottonseed oil is ranked fifth in production and consumption volume among all vegetable oils, accounting for 8% of the world's vegetable oil consumption (Lee and Fang 2015). Cottonseed meal is a source of protein for livestock, especially beef cattle, dairy cows and sheep as it provides three to six times the protein of most grains (Agricultural Marketing Resource Center 2012). The meal may be sold in the form of meal, cake, flakes or pellets. Some cottonseed meal is also used as a fertilizer for use on lawns, flower beds and gardens (Agricultural Marketing Resource Center 2012). Cottonseed hulls are used mainly as feed for livestock serving as roughage rather than as a supplement. Cottonseed hulls can also be used in petroleum refining and plastics manufacturing (Agricultural Marketing Resource Center 2012).

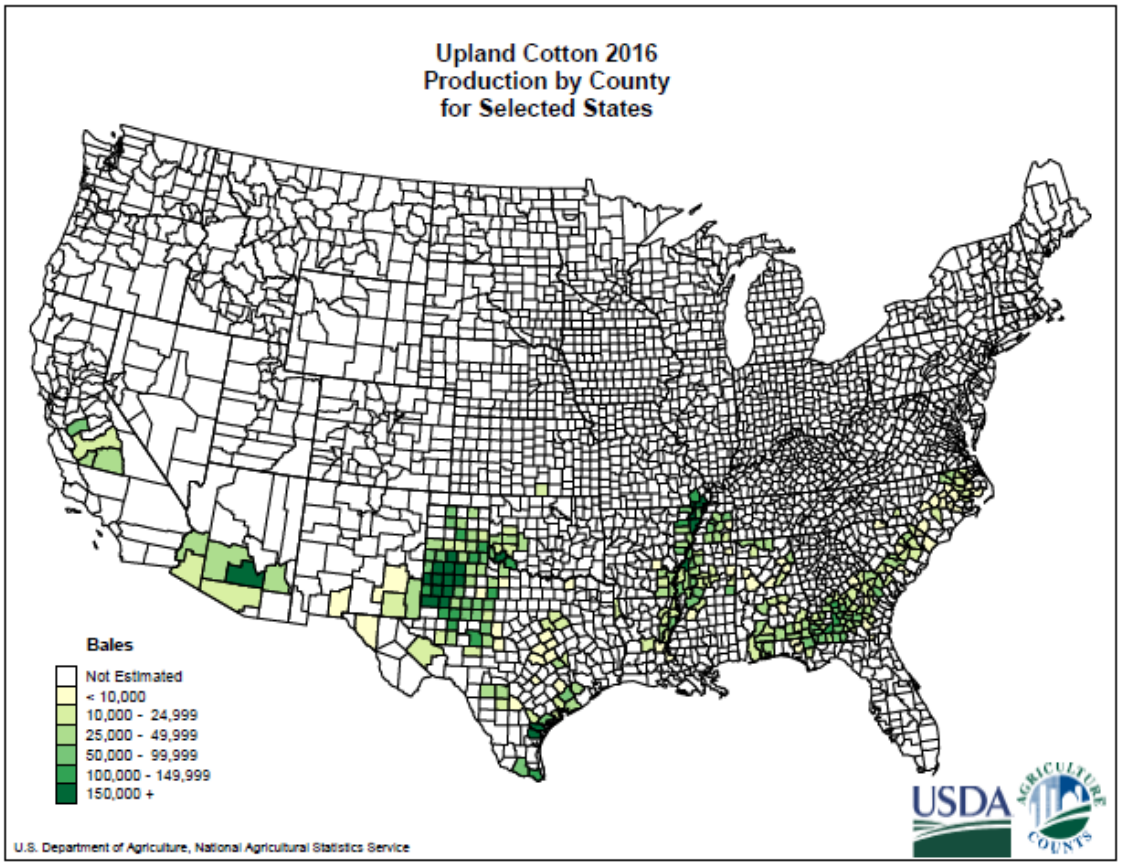

Figure 1. 2016 cotton bale production per county in the U.S. (USDA-NASS 2016).

GHB811 cotton is a dual herbicide-resistant cotton with resistance to glyphosate and isoxaflutole (IFT). Bayer has described their commercial plans for event GHB811 cotton in their petition (Bayer 2017a). Bayer plans to offer GHB811 cotton in stacked trait varieties with three additional GE-events already deregulated by USDA: T304-40 (lepidopteran resistant and glufosinate resistant), GHB119 (lepidopteran resistant and glufosinate resistant), and COT102 (lepidopteran resistant). The resulting commercial product will have various combinations of herbicide resistance to IFT, glyphosate, and glufosinate in tandem with various combinations of three independently-acting lepidopteran targeting proteins (Cry1Ab, Cry2Ae, and Vip3Aa19; Bayer 2017a).

Bayer is specifically requesting from EPA a modified label for IFT permitting its use only on 4-hydroxyphenylpyruvate dioxygenase (HPPD)-inhibitor resistant cotton varieties developed with event GHB811 cotton and not for use as a pre- or post- emergence herbicide on other GE or conventional cotton varieties that do not contain the GHB811 event. Bayer (2017a) anticipates that IFT will be labeled for pre-emergence (PRE) and early post-emergence (EPO) use patterns in GHB811 cotton. According to Bayer, the addition of IFT herbicide-resistance in GHB811

cotton will offer an alternative weed control option to help manage problem weed species. Weed species that IFT controls or suppresses in corn have been listed in the petition and Bayer extrapolates that GHB811 cotton treated with IFT would have the same weed targets (Table 9.1, p. 145; Bayer 2017a).

Cotton Event GHB811 was developed by *Agrobacterium*-mediated transformation of the conventional cotton variety Coker 312. The transformation used a vector containing *hppdPfW336-1Pa* and *2mepsps* expression cassettes conferring herbicide resistance to IFT and glyphosate, respectively (Bayer 2017a). APHIS-BRS has made 24 determinations of non-regulated status for crops with glyphosate-resistance. Of these, three of the determinations were for cotton: two had the *CP4epsps* gene from *Agrobacterium tumefaciens* strain CP4 (Monsanto 1995, 2004) and one was the double mutant *2mepsps* gene from corn inserted in Bayer cotton Event GHB614 (Bayer 2006; USDA-APHIS-BRS 2017). The breeding program diagram for the development of Event GHB811 is found in the petition (Figure 3.2, p. 20; Bayer 2017a).

Compositional, phenotypic, and agronomic analyses compared GHB811 cotton (untreated and treated with IFT and glyphosate) to its parental cotton variety, Coker 312, as well as seven conventional reference varieties (FM958, FM989, ST457, DP399, ST468, FM966 and Acala Maxxa). The compositional analyses were also compared to conventional nutrient and anti-nutrient ranges found in the ILSI database (ILSI 2016b).

Based on cotton biology (Fryxell 1979; Fryxell 1984; Wendel and Cronn 2003; OECD 2008; Wendel et al. 2010) and analytic data presented by Bayer (2017a) relevant to the development of GHB811 cotton, APHIS concludes that the use of the nonGE parental line Coker 312 in addition to the seven other reference varieties used as comparators is sufficient to determine whether Event GHB811 cotton poses an increased plant pest risk compared to its nonGE parental line and nonGE conventional cotton (USDA-APHIS-BRS 2017).

C. Description of Inserted Genetic Material, Its Inheritance and Expression, Gene Products, and Changes to Plant Metabolism

To inform the potential hazards resulting from the genetic modification and potential routes of exposure related to the inserted DNA and its expression products, APHIS assessed data and information presented in the petition related to: the transformation process; the source of the inserted genetic material and its function in both the donor organism and the GE crop event; and the integrity, stability and mode of inheritance of the inserted genetic material through sexual or asexual reproduction based on the location of the insertion (e.g. nucleus or organelle) and the number of loci inserted.

APHIS also assessed data presented in the petition on whether the genetic modification results in expression of new genes, proteins, or enzymes or changes in plant metabolism or composition in the Event GHB811 cotton relative to the nontransgenic counterpart**.** The assessment encompasses a consideration of the expressed proteins double mutant 5-enol pyruvylshikimate-3-phosphate synthase (2mEPSPS) and single mutant 4-hydroxyphenylpyruvate dioxygenase (HPPD W336) and any observed or anticipated effects on plant metabolism including, e.g. any

relevant changes in levels of metabolites, antinutrients, or nutrients in fuzzy seed derived from the GE crop event compared to those in the conventional counterpart and to its nonGE conventional varieties.

This information is used later in this risk assessment to inform whether there is any potential for plant pest vectors or sequences to cause disease or greater plant pest risks in the GE crop event; or for expression of inserted DNA, new proteins or enzymes, or changes in metabolism to affect plant pest or diseases, nontarget beneficial organisms, weediness, agricultural practices that impact pest or diseases or their management, or plant pest risks through horizontal gene flow.

Description of the genetic modification and inheritance of inserted DNA

The transformation methodology used is described in the petition (Section 3; Bayer 2017a). The Coker 312 variety was transformed using an *Agrobacterium tumefaciens*-mediated cotton hypocotyl transformation method with the disarmed binary plasmid vector pTSIH09 containing *hppdPfW336-1Pa* and *2mepsps* expression cassettes (Bayer 2017b). After co-cultivation with disarmed *A. tumefaciens*, the transformed cotton hypocotyl pieces were transferred to medium containing the antibiotic Ticarcillin to eliminate *A. tumefaciens* (Bayer 2017b). After the *A. tumefaciens* was eliminated, the T0 transformed plants were treated with tembotrione (HPPD-inhibitor herbicides) to select for the expression of the *hppdPfW336-1Pa* gene. The surviving plants were then self-pollinated to generate T1 seed. Subsequent T2 to T7 generations were produced through self-pollination and some plants were sprayed with glyphosate to ensure expression of the *2mepsps* gene at those generations. In the addition, in the development of GHB811 cotton varieties, T0 plants were crossed into a conventional commercial cotton line.

The two genes of interest inserted into GHB811 cotton are the single mutant 4-hydroxyphenylpyruvate dioxygenase (*hppdPfW336-1Pa*) gene and the double mutant 5-enol pyruvylshikimate-3-phosphate synthase (*2mepsps*) gene which encode proteins that confer resistance to the herbicides IFT and glyphosate, respectively.

The *hppdPfW336-1Pa* gene encodes for the HPPD W336 protein. The *hppdPfW336-1Pa* sequence was developed by changing the codon for amino acid glycine at position 336 to a codon for tryptophan in the wild type *hppd* gene cloned from *Pseudomonas fluorescens* strain A2 (Boudec et al. 2001). The HPPD W336 protein is functional, retaining the properties of catalyzing the transformation of 4-hydroxyphenylpyruvate into homogentisate, but is less sensitive to HPPD inhibitors than the native HPPD protein (Boudec et al. 2001). This HPPD W336 protein makes the transformed cotton resistant to HPPD inhibitors, such as isoxaflutole (Boudec et al. 2001; Bayer 2017a).

The *2mepsps* gene encodes for the 2mEPSPS protein. The *2mepsps* coding sequence was developed by introducing two point mutations to the wild-type *epsps* gene cloned from maize (*Zea mays*), resulting in the substitution of threonine by isoleucine at position 102 and the substitution of proline by serine at position 106 (Lebrun et al. 1997). These modifications confer decreased binding affinity for glyphosate, allowing it to maintain sufficient enzymatic activity in the presence of the herbicide (Funke et al. 2009).

Table 3.1 (p. 18; Bayer 2017a) lists all genetic material include in the pTSIH09 plasmid used to transform conventional cotton variety, Coker 312. Figure 2 shows the resulting T-DNA inserted into the genome of GHB811 cotton.

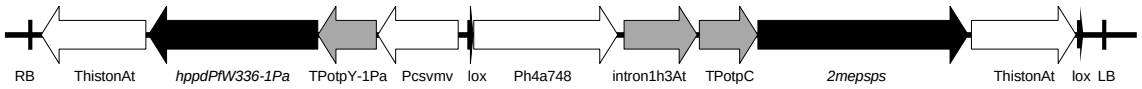

Figure 2. Linear depiction of the GHB811 cotton transgenes (from Figure 5.1, p. 24; Bayer 2017a).

The inserted DNA contains the following genetic elements:

- **Right border (RB)** repeat from the T-DNA of *Agrobacterium tumefaciens* (Zambryski 1988).
- **ThistonAt:** sequence including the 3´ untranslated region of the histone H4 gene of *Arabidopsis thaliana* (Chauboute et al. 1987).
- ***hppdPfW336-1Pa*:** coding sequence of the 4-hydroxyphenylpyruvate dioxygenase gene of *Pseudomonas fluorescens* strain A32 modified by the replacement of the amino acid glycine 336 with a tryptophan and adapted to cotton codon usage (Boudec et al. 2001).
- ***TPotpY-1Pa*:** coding sequence of an optimized transit peptide derivative (position 55 changed into Tyr), containing sequence of the RuBisCO small subunit genes of *Zea mays* and *Helianthus annuus*, adapted for cotton codon usage (Lebrun et al. 1996).
- **Pcsvmv:** sequence including the promoter region of the Cassava Vein Mosaic Virus (Verdaguer et al. 1996).
- **lox:** sequence including the 34bp recognition sequence for the Cre recombinase of bacteriophage P1 (Hoess and Abremski 1985).
- **Ph4a748:** sequence including the promoter region of the histone H4 gene of *Arabidopsis thaliana* (Chauboute et al. 1987).
- **Intron1 h3At**: first intron of gene II of the histone H3.III variant of *Arabidopsis thaliana* (Chaubet et al. 1992).
- ***TPotpC*:** coding sequence of the optimized transit peptide, containing sequence of the RuBisCO small subunit genes of Zea *mays* and *Helianthus annuus* (Lebrun et al. 1996).
- ***2mepsps:*** coding sequence of the double-mutant 5-enol-pyruvylshikimate-3-phosphate synthase gene of *Zea mays* (Lebrun et al. 1997).
- **ThistonAt:** sequence including the 3´ untranslated region of the histone H4 gene of *Arabidopsis thaliana* (Chauboute et al. 1987).
- **lox:** sequence including the 34bp recognition sequence for the Cre recombinase of bacteriophage P1 (Hoess and Abremski 1985).
- **Left border (LB)** repeat from the T-DNA of *Agrobacterium tumefaciens* (Zambryski 1988).

Bayer conducted a detailed molecular characterization of the inserted DNA and associated flanking sequences in GHB811 cotton compared to the recipient cotton line and the plasmid

vector, summarized in Figure 5.8 (p. 43; Bayer 2017a). This analysis, which included both Southern blot and DNA sequence analysis, demonstrated:

- The T-DNA inserted into the GHB811 cotton genome is present at a single locus, and contains one functional copy of the *2mepsps* and *hppdPfW336-1Pa* gene expression cassettes with truncated portions of the T-DNA right border (25 bp) and left border (24 bp) (Section 5.2, p. 33-55; Bayer 2017a);

- The inserted T-DNA was stably inherited across five breeding generations in a manner that is predictable according to Mendelian principles and consistent with insertion into a single chromosomal locus within the cotton nuclear genome (Section 5.4, p. 65-66; Bayer 2017a) (Section 5.1, p. 23-32; Bayer 2017a);

- The final product does not contain any of the backbone sequences from the plasmid pTSIH09 outside of the T-DNA region or *2mepsps* and *hppdPfW336-1Pa* expression cassette borders (Section 5.3, p. 56-64; Bayer 2017a); and,

- The T-DNA sequence inserted in GHB811 cotton is identical to the corresponding T-DNA sequence of the original donor plasmid pTSIH09 (Section 5.6, p. 66-69; Bayer 2017a).

Expression of inserted DNA, changes in gene expression, new proteins or metabolism

GHB811 plant samples were analyzed during herbicide treatment and when not treated with herbicide. The quantitation of both the 2mEPSPS and HPPD W336 proteins were done on leaf, root, pollen square, boll, whole plant and fuzzy seed samples for both fresh weight (FW) and dry weight (DW). The expression levels in all parts of the GHB811 cotton plants are found in Table 6.2 in the petition (p. 78; Bayer 2017a).

HPPD W336 Protein

HPPD proteins are ubiquitous in nature across all kingdoms: bacteria, fungi, plants and animals including mammals (Brownlee et al. 2004). The biochemical pathways in which HPPD is involved differ between plants and non-photosynthetic organisms. In bacteria and animals, it serves catabolic purposes by catalyzing the first committed step in tyrosine degradation that ultimately yields glucogenic and ketogenic products (Brownlee et al. 2004). In plants, however, it is also involved in several anabolic pathways; its reaction product homogentisate (2,5-dihydroxyohenylacetate) being the aromatic precursor of tocopherol, tocotrienols and plastoquinone that are essential to the photosynthetic transport chain and antioxidative systems (Figure 3) (Fritze et al. 2004). Specifically, tocopherols are antioxidants that play a role in plant stress tolerances (Saini and Keum 2016). Plastoquinone is a carotenoid pigment that is needed for photosynthesis (Rippert et al. 2004). When IFT is applied to a plant, it is rapidly converted into diketonitrile (DKN), which inhibits HPPD, preventing the biosynthesis of tocopherols and plastoquinone (Figure 4). IFT treatment prevents the replacement of chlorophyll after it is broken

down in sunlight, causing a bleaching effect. Bleaching symptoms first appear on leaf edges and tips at the site of new carotenoid synthesis (Johnson et al. 2002).

USDA-APHIS-BRS has deregulated one event in soybean containing an HPPD gene resistant to HPPD inhibitors (Syngenta 2012). The *hppd* gene modified for IFT resistance in the Bayer petition (2017a) was isolated from the bacterium *Pseudomonas fluorescens*, strain A32. *P. fluorescens* is a Gram-negative, rod-shaped, motile, asporogenous, aerobic bacterium. *P. fluorescens*, is ubiquitous in the environment, including soil, water and food, and is not considered a plant pest . The modified HPPD enzyme conferring tolerance to IFT herbicide has a single amino acid substitution of glycine (G) to tryptophan (W) at position 336 resulting the modified IFT-tolerant HPPD W336 protein (Boudec et al. 2001; Bayer 2017a).

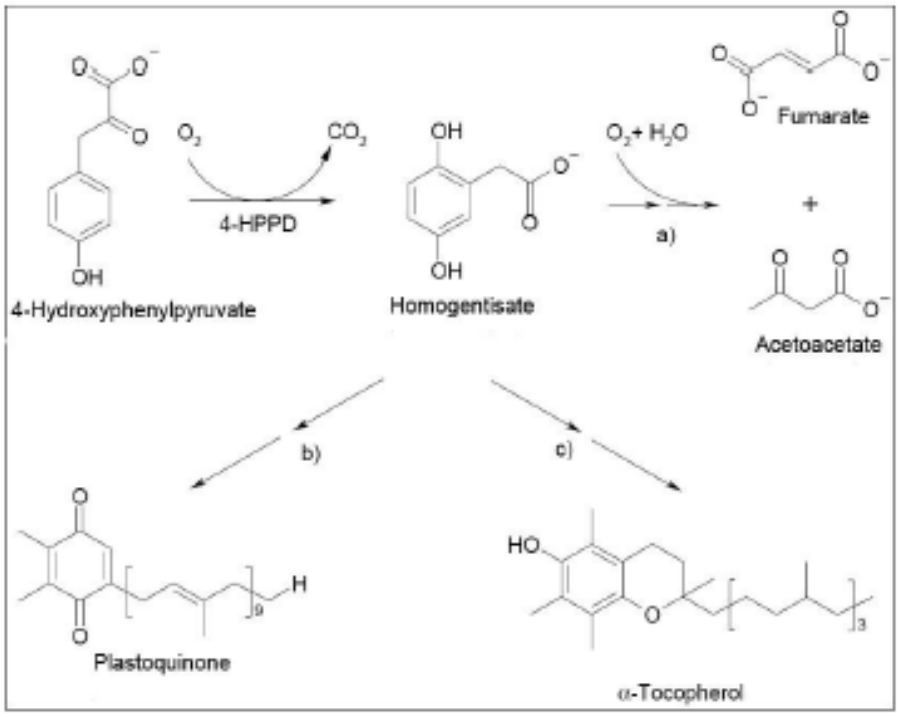

Figure 3. Biological pathway of HPPD proteins (Figure 6.2, p. 74; Bayer 2017a). The inhibition of the 4-HPPD enzyme disrupts the metabolism of the amino acid tyrosine (Wu et al. 2002).

a) Catabolism of tyrosine,

b) Biosynthesis of plastoquinone (plants),

c) Biosynthesis of α-tocopherol and tocotrienols (plants)

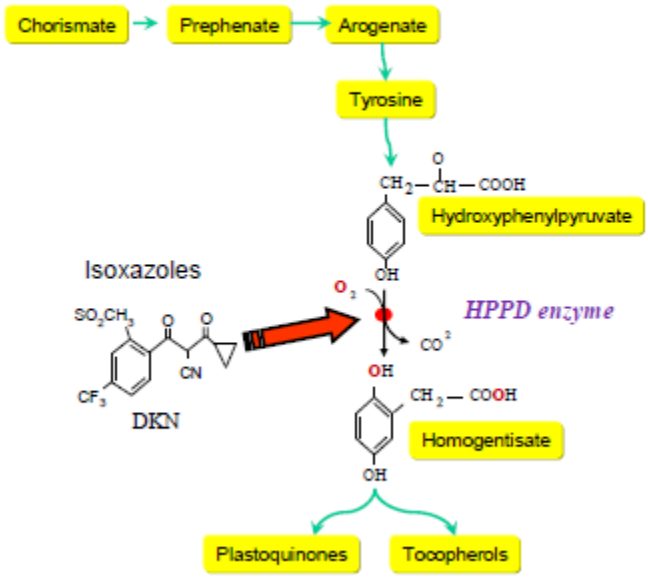

Figure 4. The interaction of HPPD and IFT's in the biochemical pathway in the secondary metabolites created by the aromatic amino acid, tyrosine (Figure 6.1, p. 73; Bayer 2017a).

HPPD protein was extracted from GHB811 plants that were either treated with herbicide or were left untreated. The plant samples came from field trial sites selected in MS, NC and TX that are representative of commercial cotton production areas (Bayer 2017a). Protein was analyzed in the leaves and roots during the 4-6 leaf stage, in leaves during the square initiation stage, in pollen during flowering, and in leaves, squares, bolls, and the whole plant two weeks after the first flower. The protein was also extracted from fuzzy seed at plant maturity (Table 6.1; p. 78; Bayer 2017a).

The highest protein expression levels were reported in leaves at the square initiation growth stage. Roots had the lowest measurable protein expression levels among all tissues tested, however, protein levels in pollen were below Lower Limit of Quantitation (LLOQ). Treated and untreated GHB811 cotton samples had values that were similar among all plant tissues for either treatment group.(Bayer 2017a).

2mEPSPS Protein

GHB811 cotton contains the *2mepsps* gene, a double mutant version of the corn *epsps* gene. Corn is not considered a plant pest.

USDA-APHIS-BRS has made 24 determinations of non-regulated status for crops with glyphosate resistance conferred by an *epsps* gene. Fifteen of the determinations were for crops containing the *CP4 epsps* gene developed by Lebrun (1997), while seven were for crops containing different mutations of the *epsps* gene including the *2mepsps* gene. The *2mepsps* gene

in GHB811 cotton contains the same genetic modification to the *epsps* gene as found in the Bayer Event GHB614 (Bayer 2006, 2017a).

All events in the 24 determinations for *epsps*-mediated glyphosate resistant crops have gone through voluntary FDA Food Safety Consultations (FDA 2017). EPSPS genes and proteins are ubiquitous in nature and are consumed in a variety of food and feed sources, including corn, from which 2mEPSPS was derived. EPSPS proteins are also naturally present in soybean, fungal and microbial food sources such as baker's yeast (*Saccharomyces cerevisiae*), all of which have a history of safe human consumption (Harrison et al. 1996; ILSI 2016a). Additional information on the safety and mechanism of action of *epsps* proteins can be found in the documentation associated with the previous 24 determinations (USDA-APHIS-BRS 2017).

In brief, the *epsps* gene encodes the 5-enolpyruvylshikimate-3-phosphate synthase enzyme, a key enzyme in the shikimate pathway. This pathway synthesizes aromatic amino acids and other aromatic compounds in plants, fungi and microorganisms. In conventionally-bred plants, the EPSPS enzyme is selectively inhibited by glyphosate, leading to the death of the plants by shutting off the synthesis of aromatic amino acids and secondary metabolites (Amrhein et al. 1980). Figure 5 shows the target in the shikimate pathway where the herbicide glyphosate prevents the production of essential aromatic compounds tyrosine (Tyr), tryptophan (Trp), and phenylalanine (Phe). The 2mEPSPS enzyme encoded by the *2mepsps* gene in Event GHB811is insensitive to glyphosate inhibition, but has retained its function in the shikimate pathway (Steinrucken and Amrhein 1980), thereby allowing the continued production of aromatic amino acids in the presence of glyphosate.

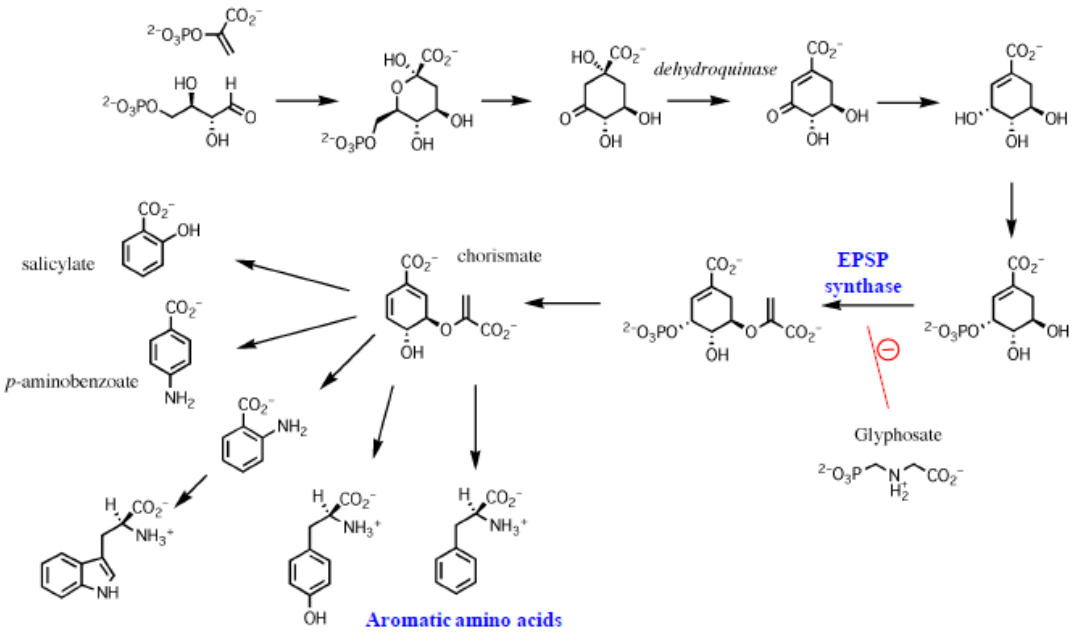

Figure 5. The shikimate pathway (Figure 6.1; Bayer 2017a).

2mEPSPS protein was extracted for both herbicide-treated GHB811 and non-treated GHB811 at varying growth stages for leaf, root, square, boll, whole plant, and fuzzy seed, as was done with the HPPD W336 protein described above (p. 76; Table 6.1; Bayer 2017a). The plant tissue samples came from the same field trial sites as the HPPD W336 samples in MS, NC and TX (Bayer 2017a).

Leaves at the square initiation stage and two weeks after the first flower stages had the highest mean for 2mEPSPS protein expression levels (Table 6.2; p.78; Bayer 2017a). Root and pollen demonstrated the lowest mean 2mEPSPS protein expression among all plant tissues reported (Table 6.2; p. 78; Bayer 2017a). The mean 2mEPSPS concentrations for untreated and treated pollen were similar for both groups (Bayer 2017a).

Compositional analysis

Compositional analyses were done on GHB811 fuzzy seed to determine if there were any relevant changes in levels of metabolites, antinutrients, or nutrients compared to those in the conventional counterpart and to its nonGE conventional varieties.

Detailed compositional and nutritional comparisons of GHB811 cotton, nonGE parental Coker 312 control and nonGE reference varieties were conducted on seed collected from eight sites across the U.S. in 2014 and 2015 (Table 7.1, p. 106; Bayer 2017a). The analysis included moisture, protein, fat, ash, carbohydrates, amino acids, fatty acids, fibers, anti-nutrients, vitamin E isoforms, minerals and vitamins. Composition analysis and the comparative assessments are found in Tables 7.5-7.9 (p. 114-118; Bayer 2017a).

Comparison of proximates[4] and fiber in fuzzy seed are shown in Table 7.5 (p. 114; Bayer 2017a). The crude protein in both the herbicide-treated and untreated GHB811 cotton was slightly but statistically lower compared to nonGE parental variety Coker 312, however, the means were within the range of the nonGE reference varieties and thus the difference is not biologically relevant. Neutral detergent fiber in herbicide-treated GHB811 cotton was slightly but statistically higher compared to the nonGE parental Coker 312 variety, however, the means were within the range of the nonGE reference varieties and thus the difference is not biologically relevant. Percent moisture, ash, carbohydrates, crude fat, acid detergent fiber and total dietary fiber had no statistical differences between the nonGE Coker 312 parental variety and herbicide-treated and untreated GHB811.

The amino acid levels in fuzzy seed can be found in Figure 7.6 (p. 115; Bayer 2017a). No statistically significant differences were found in the three essential amino acids produced via the shikimate pathway, tyrosine, tryptophan and phenylalanine, in untreated or herbicide treated GHB811 cotton compared to the nonGE Coker 312 variety. Small but statistically significant reductions in methionine and cysteine were found in treated GHB811 cotton, but the levels were still well within the ranges found in the reference varieties and in the ILSI crop composition database (ILSI 2016b). No statistically significant differences were shown in the remaining amino acid concentrations and all were found to be within the ranges of the reference varieties.

[4] Proximates refer to moisture, ash, carbohydrates, crude fat, and crude protein which are expressed as a percentage of the content in the sample.

Statistically significant differences were found in fuzzy seed from GHB811 cotton compared to nonGE Coker 312 in three fatty acids (palmitoleic acid slightly increased, stearic acid slightly decreased, arachidic acid slightly decreased) as seen in Table 7.7 (p. 116; Bayer 2017a), but their levels were still well within the ranges found in the reference varieties and in the ILSI crop composition database (ILSI 2016b).

Tocopherols are substances produced by plants from homogentisate, the product of HPPD, and play an important role as antioxidants. The amount and composition of tocopherols are regulated in part by biotic and abiotic factors surrounding the plants, such as developmental stage, stresses and nutrient availability (Tsegaye et al. 2002). Alpha-tocopherol is an isoform with the highest amount of vitamin E activity and is an essential dietary component for mammals (Ujiie et al. 2005). The level of α-tocopherol in fuzzy seed from herbicide-treated and untreated GHB811 cotton was slightly, but statistically lower than in fuzzy seed from the nonGE Coker 312 parental variety (See Table 7.8, p. 117). No statistically significant differences in α-tocopherol were seen between the herbicide-treated GHB811 cotton and untreated GHB811 cotton. All α-tocopherol levels in the nonGE Coker 312, herbicide treated and untreated GHB811 comparisons were above the mean range of the reference varieties but were well within the levels of tocopherols found in the ILSI database (ILSI 2016b), thus making the statistical differences not biologically relevant.

Gossypol is a yellow phenolic pigment that is an anti-nutrient naturally found in cottonseed meal. Gossypol toxicity limits cottonseed use in animal feed. The gossypol levels were tested in fuzzy seed from the nonGE Coker 312 variety, herbicide treated and untreated GHB811 varieties and reference varieties as seen in Table 7.9 (Bayer 2017a). The gossypol levels were lower (statistically different) in both herbicide treated and untreated GHB811 cotton compared to the nonGE Coker 312 variety, but were well within the limits of the reference variety ranges and the reported range found in the ILSI database (ILSI 2016b; Bayer 2017a).

Based on the data presented by the petitioner on the composition of key nutrients, anti-nutrients, and secondary metabolites in cotton fuzzy seed for both herbicide-treated and untreated GHB811 cotton, it is reasonable to conclude that neither the insertion of the glyphosate and IFT herbicide-resistance genes nor their respective gene products have a meaningful impact on the composition of cotton fuzzy seed derived from GHB811 cotton compared to other commercial cotton varieties. Based on all the above noted considerations, APHIS concludes that GHB811 cotton poses no more of a plant pest risk from new gene products or changes to plant metabolism or composition than conventional cotton varieties.

D. Potential Plant Pest and Disease Impacts

APHIS assessed whether potential plant pest or disease impacts are likely to result from the transformation process, from DNA sequences from plant pests, or from any other expression products, new enzymes, proteins or changes in plant metabolism or composition in GHB811 cotton that are known or anticipated to cause disease symptoms, or to affect plant pests or diseases or plant defense responses (as identified from the previous section). APHIS also assessed or whether GHB811 cotton is likely to have significantly increased disease and pest susceptibility based on data and observations from field trials and/or laboratory experiments on specific pest and disease damage or incidence and any agronomic data that might relate to such

damage. Impacts or changes are assessed to determine if they would (1) affect the new GE crop and/or result in significant introduction or spread of a damaging pest or disease to other plants; (2) result in the introduction, spread, and/or creation of a new disease; and/or (3) result in a significant exacerbation of a pest or disease for which APHIS has a control program. Any increase in pest or disease susceptibility is evaluated with respect to the context of currently cultivated varieties, the ability to manage the pest or disease, and the potential impact on agriculture.

Plant Protection and Quarantine (PPQ) is an APHIS program that safeguards agriculture and natural resources from the entry, establishment, and spread of animal and plant pests and noxious weeds into the United States of America; and supports trade and exports of U.S. agricultural products. PPQ responds to many new introductions of plant pests to eradicate, suppress, or contain them through various programs in cooperation with state departments of agriculture and other government agencies. These may be emergency or longer term domestic programs that target a specific pest. A variety of insect, plant disease, mollusk, nematode or weed programs exist (USDA-PPQ 2017). PPQ has programs targeting two cotton pests: boll weevil and pink bollworm. Boll weevil has been eradicated in the U.S. except for several counties in the southern tip of TX (USDA-PPQ 2014). Nearly all 17 cotton states have local regulations concerning the boll weevil (USDA-PPQ 2017). A report by the National Cotton Council stated the last pink bollworm moth was captured in a pheromone trap in 2013, although monitoring of Western Texas and New Mexico continues in order to verify eradication efforts have been successful (National Cotton Council 2018). More information on these cotton pests can be found on USDA's website under Plant Health, Pests and Diseases (USDA-PPQ 2017).

A number of other insects also feed on cotton. From 2015- 2016, the most important cotton pests affecting yield were thrips (Thripidae), cotton fleahopper (*Pseudatomoscelis seriatus*), aphids (Aphididae), bollworm/budworm (*Helicoverpa zea* and *Heliothis virescens*), stink bugs (Pentatomidae), and *Lygus* species (Mississippi State University 2016). Of the various diseases of cotton, those that have most affected yield in 2016 are *Phymatotrichum* root rot (*P. omnivorum*), the boll rots (*Rhizopus*), the fungal seedling diseases (primarily *Rhizoctonia solani, Pythium spp*, and *Fusarium spp*), and all nematodes, primarily *Meloidogyne spp*. (National Cotton Council 2016).

Cotton is not itself a plant pest in the U.S. The introduced genetic elements and the expression of the gene products and their functions in Event GHB811 have been summarized above. Plant pathogen sequences inserted into Event GHB811, i.e. the T-DNA border sequences from *Agrobacterium tumefaciens* and the regulatory promoter sequences from Cassava Vein Mosaic Virus (CVMV), do not cause plant disease. The plant pathogen sequences comprise only non-coding sequences and do not result in the production of infectious agents or disease symptoms in plants. The Coker 312 cotton variety was transformed using disarmed-*A. tumefaciens* to create GHB811 cotton; disarmed-*A. tumefaciens* vector lacks T-DNA sequences from Ti (Tumor-inducing) plasmids normally responsible for the formation of crown gall tumors upon *A. tumefaciens* infection (Hellens and Mullineaux 2000; Bayer 2017b). The antibiotic Ticarcillin was used by Bayer to eliminate *A. tumefaciens* after transformation (Bayer 2017b).

Bayer collected observations of cotton diseases and insect pests from field test studies conducted at 15 locations with replicates of four plots per location (Bayer 2017a). These locations cover a diverse range of environmental conditions representative of most commercial cotton production areas and locations where GHB811 is expected to be grown. The agronomic practices used to prepare and maintain each field site were characteristic of each respective region. Bayer categorized the degree of abiotic, biotic and insect stress in four different growth stages at each of the 15 sites: the two leaf-stage to floral initiation (BBCH 12 to 52), floral bud enlargement to peak bloom (BBCH 54 to 65), flowering (BBCH 61 to 69), and boll maturation (BBCH 81 to 89). The descriptions of the categorical stress rating scales used are found on pages 127-128 of the petition (Bayer 2017a). Ratings were also made at plant maturity for plant lodging. Results of the ratings are summarized in Table 8.7 and the data are analyzed in Table 8.8 (Bayer 2017a). Bayer used Cochran-Mantel-Haenszel (CMH) statistics for the categorical parameters summarized in Table 8.7. Bayer also included the mean ± standard deviation (SD) in Table 8.7 to help with the interpretation, however the means were not used to conduct CMH test. Statistical significance was evaluated at $p<0.05$ level. The CMH statistical model is explained in detail on p. 128-129 in the petition (Bayer 2017a).

The CMH statistics found a significant difference in disease stress at the flowering growth stage. The parental nonGE Coker 312 had a statistically higher disease level than the GHB811 cotton (p. 133; Bayer 2017a). No other significant differences in disease or insect stress were seen at any other stage. Table 8.9 shows the disease and insect pests observed at each of the 15 field trials; most pests were observed evenly across all field trial entries and plots at any given trial site (p. 135-137; Bayer 2017a). In addition, no difference in plant lodging, which can indirectly indicate effects of disease or insect pests, was observed. Thus, with the single exception just noted, the introduced genes did not significantly alter the observed insect pest infestation, disease occurrence or resulting damage to GHB811 cotton compared to the control line. As discussed earlier there were no significant changes in GHB811 cotton composition that would render GHB811 cotton more susceptible to pests and diseases over its control or reference cotton varieties (Bayer 2017a). The observed agronomic traits also did not reveal any significant changes that would indirectly indicate that GHB811 cotton is or could be relatively more susceptible to pests and diseases over control or reference cotton varieties (Bayer 2017a). Thus GHB811 cotton is unlikely to be more susceptible to plant pathogens and insect pests than conventional cotton. For this reason, GHB811 cotton is unlikely to differ from conventional cotton in its ability to harbor or transmit plant pathogens or pests and cause indirect plant pest effects on other agricultural products.

E. Potential Impacts on Nontarget Organisms Beneficial to Agriculture

GHB811 cotton is not engineered for pest resistance, thus there are no ‘target’ species, and thus no ‘nontarget’ species either. APHIS assessed whether exposure to or consumption of GHB811 cotton would have a direct or indirect adverse impact on species beneficial to agriculture**.** Organisms considered were representatives of the species associated with production of the regulated crop in the agricultural environment. The assessment includes an analysis of data and information on the GHB811 cotton compared to the non-GE counterpart (or other comparators) for any biologically relevant changes in the phenotype or substances produced (e.g. proteins, nutrients, or antinutrients) which may be novel or expressed at significantly altered amounts that

are associated with impacts on organisms beneficial to agriculture, and/or any observations of beneficial organisms associated with the plants.

As described above in Section C "*Expression of inserted DNA, changes in gene expression, new proteins or metabolism*", no biologically significant changes in expression of nutrients and antinutrients were detected in the compositional data (Tables 7.5-7.9; Bayer 2017a). Field trial data comparing GHB811 cotton to its parental control and reference varieties did not show any differences between comparators in the responses to biotic or abiotic stressors that would indicate a potential to impact beneficial organisms associated with the plants. Phenotypic and agronomic analyses compared GHB811 cotton (untreated and treated with IFT and glyphosate) to its parental cotton variety, Coker 312 and seven reference varieties (Table 8.6, p. 122; Bayer 2017a). When GHB811 cotton was compared to its parental nonGE variety and reference varieties a few significant differences were seen in their phenotypic or agronomic properties (see section F below), but they were not biologically relevant except for the resistance to IFT and glyphosate herbicide in the herbicide-treated groups.

Both the 2mEPSPS and HPPD W336 proteins expressed in GHB811 cotton were determined to be identical to the microbially-produced proteins from recombinant *Escherichia coli* based on protein gel electrophoresis, western blot analysis, glycosylation analysis, mass spectroscopy with peptide mapping, and N-terminal sequence analysis (Bayer 2017a).

In vitro studies with the bacterially-produced 2mEPSPS and HPPD W336 proteins were done on digestibility in simulated gastric and intestinal fluid, heat stability, homology with known allergens and acute toxicity. Both proteins were degraded very rapidly in human simulated gastric fluid within 30 seconds of incubation in presence of pepsin, at pH 1.2. Both were also degraded very rapidly in intestinal fluid with no fragment protein visible within 30 seconds of incubation in presence of pancreatin, at pH 7.5. (p. 98-99 and p. 101, respectively; Bayer 2017a).

The overall identity search showed no biologically relevant identity between 2mEPSPS and HPPD W336 proteins and any known allergenic proteins or toxins (p. 103-105; Bayer 2017a).

Using the microbially-produced 2mEPSPS and HPPD W336 proteins, acute toxicity studies in C57BL/6J mice demonstrated no mortalities, no treatment-related clinical signs, no effects on the body weight and food consumption parameters as well as no macroscopic changes at necropsy after an acute oral administration at 2000 mg/kg body weight (p. 105; Bayer 2017a). The levels of the 2mEPSPS and HPPD W336 proteins measured in event GHB811 cotton tissues were exponentially lower than the doses tested in the acute oral mouse study.

Therefore, based on the above analysis of compositional data, agronomic and phenotypic observations, and food and feed safety studies (Bayer 2017a), APHIS concludes that exposure to and/or consumption of the GE plant are unlikely to have any adverse impacts to organisms beneficial to agriculture.

F. Potential for Enhanced Weediness of GHB811 Cotton

APHIS assessed whether GHB811 cotton is likely to become more weedy (i.e. more prevalent, competitive, damaging or difficult-to-control in situations where it is not wanted) than the

nontransgenic progenitor from which it was derived, or other varieties of the crop currently under cultivation. The assessment considers the basic biology of the crop, the situations in which crop volunteers or feral populations are considered weeds, and an evaluation of GHB811 cotton compared to the nontrangsenic progenitor and the other reference varieties evaluated under field (and/or lab) conditions characteristic for the regions of the U.S. where GHB811 cotton is intended to be grown. Characteristics related to establishment, competiveness, reproduction, survival, persistence and/or spread that could influence weediness and the ability to manage the crop as a weed were evaluated. For cotton, such characteristics include seed dormancy and germination, rate of growth, plant height, seed yield and percent ground cover, and differences in response to biotic (insects, arthropods, microbial diseases) and abiotic (environmental parameters such as wind events, heat, hail, moisture, drought, excessive rain, and nutrient deficiencies) stressors. The assessment also considers whether the engineered trait affects methods of control for the crop in situations where it is managed as a weed or volunteer in subsequent crops or in feral populations.

Upland cotton (*G. hirsutum*) is a domesticated perennial grown as an annual crop that is not generally persistent in unmanaged or undisturbed environments without human intervention. It possesses few of the characteristics common to plants that are successful weeds (Baker 1965; Keeler 1989) and is not considered to be a serious or common weed in the United States. It is not listed as a weed in the major weed references (Crocket 1977; Holm et al. 1979; Muenscher 1980), nor is it present on Federal or State lists of noxious weed species (USDA-NRCS 2017). Cotton can become locally feral or naturalized in suitable areas, such as Hawaii, and Puerto Rico (Fryxell 1979; Coile and Garland 2003; USDA-NRCS 2014). Modern cultivars are not frost tolerant and do not survive freezing winter conditions, do not produce abundant or long-lived seeds that can persist or lie dormant in soil, do not exhibit vegetative propagation or rapid vegetative growth, and do not compete effectively with other cultivated plants (OECD 2008). In areas where winter temperatures are mild and freezing does not occur, cotton plants can occur as volunteers in the following growing season (Keeling et al. 2009; Thompson and Tepfer 2010; Baughman 2011; Fromme et al. 2011; Morgan et al. 2011b; Morgan et al. 2011a; Charles et al. 2013). However, these volunteers can be easily controlled by herbicides or mechanical means.

Seed dormancy is a characteristic that is often associated with plants that are considered weeds. Lab studies found no significant differences in germination (as an indicator of dormancy) of GHB811 cottonseed compared with nontransgenic control cottonseed under warm (30°C) and cool (10°C) conditions (Tables 8.10 & 8.11; p. 138; Bayer 2017a).

Bayer collected agronomic data relevant to weedy traits (e.g. final stand count, number of seeds per boll, and number of bolls per plant) from 15 field experiments over eight states across the United States during the 2014 and 2015 growing seasons (Bayer 2017a). GHB811 cotton with and without trait-specific herbicide application were compared to non-treated Coker 312 and three conventional reference varieties at each site (a total of 7 reference varieties were used across all sites). The data from the reference varieties were combined to establish 99% tolerance intervals for the various traits assessed (Bayer 2017a). The data for the measured parameters relevant to weediness as mentioned above are presented in Table 8.6 in the petition (p. 130-131; Bayer 2017a).

There was a statistical difference observed between nonGE Coker 312 and untreated GHB811 cotton for the final stand count. The nonGE Coker 312 had a slightly higher stand count than the GHB811 without herbicide treatment as well as the herbicide-treated GHB811 (although the latter difference was not statistically significant). However, this difference is not biologically relevant since the stand counts fell well within the reference variety range. There were no statistical differences between nonGE Coker 312 and treated or untreated GHB811 cotton in the percent ground cover, plant height, time or heat units to 10% flower, the number of seeds per boll, the number of bolls per plant, or the 100 seed weight (p. 130-131; Bayer 2017a). As discussed in Section D, with one minor exception, no differences in disease or insect pest susceptibility or in response to abiotic stress were observed between GHB811 cotton and nonGE Coker 312 and reference varieties.

Given these data, the herbicide-resistance traits conferred by the *hppd W336* and *2mepsps* genes are very unlikely to provide GHB811 cotton with a selective advantage in unmanaged ecosystems. However, the herbicide-resistance traits could complicate efforts to control volunteer cotton in settings where glyphosate or IFT are being applied for weed control, such as in subsequent cotton or rotation crops (Roberts et al. 2002; Fannin 2010; Ledbetter 2011). Although cotton volunteers typically do not reduce crop yield, they can act as reservoirs for insect pests of cotton (York et al. 2004). However, both mechanical means (tillage) and a variety of other herbicide treatments are available for control of volunteer cotton in such circumstances (Thompson and Steckel 2008; Keeling et al. 2009; Morgan et al. 2011b; Morgan et al. 2011a). Therefore, excepting glyphosate and IFT, GHB811 cotton is expected to be sensitive to the same herbicides as other cotton varieties.

Based on the agronomic field data and literature survey concerning weediness potential of the crop, GHB811 cotton is unlikely to persist as a troublesome weed or to have an impact on current weed management practices. Furthermore, extensive post-harvest monitoring of field trial plots planted with the GE crop event under USDA-APHIS notifications or permits did not reveal any differences in survivability or persistence relative to other varieties of the same crop currently being grown. These data suggest that GHB811 cotton is no more likely to become a weed than conventional varieties of the crop. GHB811 cotton volunteers can be managed using a variety of currently available methods and alternative herbicides such as desiccants, defoliants, and growth regulators (Appendix 4, p. 69 in Fernandez-Cornejo J. et al. 2014).

G. Potential Impacts on the Weediness of Any Other Plants with which GHB811 Cotton Can Interbreed

Gene flow is a natural biological process with significant evolutionary importance. A number of angiosperm taxa are believed to be derived from hybridization or introgression between closely related taxa (Grant 1981; Rieseberg and Wendel 1993; Soltis et al. 1993; Hegde et al. 2006), and even in the existing floras, the occurrence of hybridization or introgression is reported to be widespread (Stace 1987; Rieseberg and Wendel 1993). It has been a common practice by plant breeders to artificially introgress traits from wild relatives into crop plants to develop new cultivars (Khoury et al. 2013). However, gene flow from crops to wild relatives is also thought of as having a potential to enhance the weediness of wild relatives, as observed in rice, sorghum,

sunflower and a few other crops (see Table 1 in (Ellstrand et al. 1999). This topic is covered in two sections: 1) the potential for gene flow, hybridization and introgression from the GE crop event to sexually compatible relatives, including wild, weedy, feral or cultivated species in the United States and its territories, and 2) if so, the risk potential with respect to weediness of those taxa based on the phenotypic changes that have been observed in the engineered plants.

Potential for gene flow, hybridization and gene introgression

Two cultivated and two wild species of cotton grow in the U.S. and its territories. *G. hirsutum* (Upland cotton) is the most widely cultivated species, comprising 97% of the U.S. cotton planted in 2016 (USDA-ERS 2017a). The vast majority of Upland cotton is cultivated in the Cotton Belt, which stretches across the southern U.S. from Virginia to California (USDA-ERS 2017a). Small amounts are also grown in Puerto Rico for breeding and seed production purposes (Bayer 2017a). In addition to cultivated varieties, naturalized or native populations[5] of *G. hirsutum* grow in Florida, Puerto Rico, and the Virgin Islands, while naturalized populations grow in some of the Hawaiian Islands (Fryxell 1979; Lee 1984; Wagner et al. 1990; Coile and Garland 2003; Wagner et al. 2012; USDA-NRCS 2014; Lee and Fang 2015; USDA-NRCS 2017; Wunderlin et al. 2017).

The second cultivated species, *G. barbadense* (Pima or Egyptian cotton), is grown in Arizona, California, New Mexico, and Texas, but no longer widely grown as an agricultural commodity in Hawaii (Pleasants and Wendel 2005). Naturalized populations of *G. barbadense* grow in Puerto Rico, the Virgin Islands and most of the major Hawaiian Islands (Wagner et al. 1990; USDA-NRCS 2014, 2017). Two wild species of cotton are native to the United States, *G. thurberi* and *G. tomentosum*, which grow in Arizona and Hawaii respectively (USDA-NRCS 2014, 2017). *G. hirsutum* is tetraploid and thus effectively incompatible with diploid species such as *G. thurberi.* Plants from these two groups do not normally hybridize and produce fertile offspring in natural settings, and experimental crosses are difficult (OECD 2008). In contrast, *G. hirsutum* is sexually compatible with the tetraploids *G. barbadense* and *G. tomentosum* and can form viable and fertile progeny with both species (Brubaker et al. 1993; Saha et al. 2006; OECD 2008). Thus, unassisted outcrossing and gene introgression could potentially occur in areas where these species are co-located.

Wind dispersal of cotton pollen is negligible because of its large size and self-adherent properties (McGregor 1976; OECD 1993, 2008). However, cross-pollination between cotton species can occur through the activity of pollinating insects (McGregor 1976; Van Deynze et al. 2005; OECD 2008). For transgene introgression from GHB811 cotton to occur there would have to be spatial proximity between GHB811 cotton and the recipient variety or species, overlap in their flowering phenology, and overlap in their pollinators (Pleasants and Wendel 2005). In general, the extent of transgene introgression will depend on the species pool, preferences, and abundance of pollinators, which can vary according to region, location, season, time of day, and use of insecticides. In addition, transgene introgression will decrease with increasing geographic

[5] A "native" plant is one that has grown in a particular region or ecosystem for hundreds or thousands of years. A "naturalized" plant is one that does not need human help to reproduce and maintain itself over time in an area where it is not native USDA-NRCS. 2014. Plants Database: *Native, Invasive, and Other Plant-Related Definitions.* Retrieved from http://plants.usda.gov Last accessed 09/07/2017.

distance between the source and receiver populations and physical barriers; and intermediate pollinator-attractive plants can reduce the potential for pollen movement (Green and Jones 1953; McGregor 1976; Umbeck et al. 1991; Van Deynze et al. 2005; Zhang et al. 2005; OECD 2008). Additional information on the biology of cotton can be found within the OECD cotton consensus document (OECD 2008).

Because of eradication intended to control the pink bollworm, native and feral populations of *G. hirsutum* have become very rare. It has been listed as endangered by the state of Florida (USDA-FS 2017). Although remaining populations of *G. hirsutum* grow in Southern and Central Florida, their northernmost reported location (Gilchrest County, FL) is separated by over 120 miles from the nearest commercial cotton production areas in the Florida panhandle (Calhoun County, FL) (USDA-NASS 2017b; Wunderlin et al. 2017). Thus, outcrossing from GHB811 cotton to native and feral populations of *G. hirsutum* in Florida is highly unlikely.

In contrast, *G. hirsutum* is cultivated in many areas where *G. barbadense* is also grown (USDA-NASS 2015). In addition, as noted above, native and/or naturalized populations of both species are present in Hawaii, Puerto Rico, and the Virgin Islands. Although cultivated varieties of both species are largely self-pollinated, insect-mediated cross-pollination can occur both within and between the species (Brubaker et al. 1993; Van Deynze et al. 2005; Llewellyn et al. 2007; OECD 2008; Van Deynze et al. 2011). Bumble bees (*Bombus* spp.), *Melissodes* and *Halictus* bees, honey bees (*Apis mellifera*), and *Scolia* wasps are the primary pollinators (McGregor 1976).

Published studies report that there has been relatively little gene introgression from *G. hirsutum* into native or naturalized *G. barbadense* in Central America and the Caribbean, despite the fact that *G. barbadense* has been grown in the presence of the predominant *G. hirsutum* since prehistoric times (Fryxell 1979). In contrast, introgression from *G. barbadense* to native or naturalized *G. hirsutum* in these areas has been relatively common (Wendel et al. 1992; Brubaker et al. 1993). Various mechanisms have been suggested to account for this asymmetry (Percy and Wendel 1990; Brubaker et al. 1993; Jiang et al. 2000; OGTR 2008). While none of these mechanisms leads to complete isolation between the two species, the reported asymmetry in gene flow suggests that gene introgression from cultivated *G. hirsutum* varieties such as GHB811 cotton to native or naturalized *G. barbadense* should be rare.

However, gene introgression from cultivated *G. hirsutum* to cultivated *G. barbadense* may be more likely, since gene flow between cultivated varieties of these species appears to occur with the opposite asymmetry from that observed between native or naturalized varieties (Wendel et al. 1992; Brubaker et al. 1993; Van Deynze et al. 2011). The mechanism underlying this reversal in the directionality of gene flow accessions is not known. Nonetheless, outcrossing rates from GHB811 cotton to cultivated *G. barbadense* are still likely to be low. For instance, Van Deynze (2005) reported that Upland/Pima hybrid plants have been observed at a rate of 0.01% in fields sown with seeds of cultivated varieties that were obtained from production fields separated by at least 800 meters.

With regard to *G. tomentosum*, natural populations of this species are found on all Hawaiian Islands except Hawaii; the species is dominant on Kohoolawe and several sizable populations are found on Oahu and Maui. Populations are located on the drier, leeward coastal plains of the

islands at low elevations, which are also the areas that are primarily used for agriculture (Pleasants and Wendel 2005).

The flowering period for *G. tomentosum* corresponds to the end of the rainy season; it may begin as early as January, with peak flowering occurring in April and May, and may extend through August in a very wet year (Pleasants and Wendel 2010). Thus, any cultivated cotton that blooms between January and August could potentially overlap with *G. tomentosum*. Previously, it was thought that peak anthesis and receptivity in *G. tomentosum* occurs at dusk, whereas in *G. hirsutum* the flowers open in the morning and whither by evening (OECD 2008). However, Pleasants (2010) found that *G. tomentosum* flowers also open in the morning, dehisce rapidly, and begin to senesce by late afternoon. These results suggest that there is substantial overlap in flowering phenology between *G. hirsutum* and *G. tomentosum*.

Spontaneous self-pollination is rare in *G. tomentosum*, perhaps due to the structure of its flowers. Instead, the species appears to rely on the action of pollinators (Pleasants and Wendel 2005; Münster and Wieczorek 2007). It was previously thought that moths were the only insects that pollinated *G. tomentosum*, and thus that there was little overlap with pollinators of *G. hirsutum* (Pleasants and Wendel 2005; OECD 2008). However, more recent studies have shown that *G. tomentosum* is pollinated by honeybees and carpenter bees, which are among the species that also pollinate commercially grown *G. hirsutum*. In addition, both of these pollinators are long-distance foragers; for instance, honeybees may forage up to 6 – 10 miles from their nest (Pleasants and Wendel 2010).

Thus, in addition to overlap in flowering phenology, there is overlap in pollinators between *G. tomentosum* and *G. hirsutum*. However, no hybrids between *G. hirsutum* and *G. tomentosum* have been identified to date, although only a relatively small number of accessions and marker loci have been examined (DeJoode and Wendel 1992). Moreover, *G. hirsutum* has not been grown as an agricultural commodity in Hawaii for decades, and APHIS has no information suggesting that seed companies use the Hawaiian Islands as a winter nursery.

Expression of the 2mEPSPS and HPPD W336 proteins do not cause any major changes in the phenotype of cotton plants other than to confer resistance to the herbicides glyphosate and IFT. Thus, the introduced genetic material is unlikely to cause an increased rate of outcrossing of GHB811 cotton relative to non-transgenic varieties. Should outcrossing from GHB811 cotton to *G. barbadense* or *G. tomentosum* occur, transgene introgression would still require the establishment of hybrid progeny followed by persistence of the transgene through self-crossing or back-crossing into the recipient species in subsequent generations.

The low level of introgression from *G. hirsutum* to native or naturalized *G. barbadense* observed in the Caribbean and the phenomenon of hybrid breakdown[6] suggests that transgene introgression from GHB811 cotton to native or naturalized *G. barbadense* can occur but is likely to be rare (Fryxell 1979; Jiang et al. 2000; OGTR 2008; Fang et al. 2013). In the absence of herbicide treatment, the transgenic material in GHB811 cotton is unlikely to confer a selective advantage on any hybrid progeny that may result from outcrossing. Thus, the transgenes present in GHB811 cotton are unlikely to increase the rate of successful transgene introgression from

[6] "Hybrid breakdown" is the poor viability or lethality in F1 hybrids between species.

GHB811 cotton into native or naturalized *G. barbadense* populations relative to the rate of gene introgression from conventional cultivars.

Transgene introgression from GHB811 cotton to cultivated *G. barbadense* can also occur but is also likely to be rare since cultivated *G. barbadense* is regularly harvested. While the likelihood of transgene movements to *G. barbadense* is likely greater with cultivated varieties than with native or naturalized *G. barbadense*, such movements would tend to involve plants producing seeds intended for processing rather than planting because seed production fields are isolated from commercial fields. Seed production isolation standards will help ensure that any movement of transgenes into seed production fields will remain at very low levels (Van Deynze et al. 2005; AOSCA 2012). The transgenes present in GHB811 cotton are unlikely to increase the rate of successful transgene introgression from GHB811 cotton into cultivated *G. barbadense* relative to the rate of gene introgression from conventional cultivars.

Finally, introgression into *G. tomentosum* in Hawaii is also likely to be rare, both because of barriers to introgression (Percy and Wendel 1990; Brubaker et al. 1993; Jiang et al. 2000; OGTR 2008), and because there is no commercial cotton production on these islands (USDA-NASS 2017a). If any Upland cotton is grown in the Hawaiian Islands, it is grown at a very small scale and outcrossing to *G. tomentosum* is unlikely to occur. Should outcrossing nonetheless occur, transfer of the transgenes present in GHB811 cotton would not be expected to confer a selective advantage on the hybrid progeny or to reduce hybrid breakdown, which would be expected to eliminate introgressed genes from the *G. tomentosum* population. Thus, the transgenes present in GHB811 cotton are unlikely to increase the rate of successful transgene introgression from GHB811 cotton to *G. tomentosum*.

In summary the available evidence indicates that there is a low potential for introgression of transgenic material from GHB811 cotton to *G. tomentosum* or to native or naturalized *G. barbadense*. There is no evidence that any of the genetic elements used in GHB811 cotton would increase the rate of outcrossing or gene introgression of GHB811 cotton relative to non-transformed cotton.

Potential for enhanced weediness of recipients after hybridization and/or introgression

As discussed in the previous section, the genetic material introduced into GHB811 cotton does not confer or enhance weedy characteristics of cultivated Upland cotton. There is no reason to believe that it would do so in naturalized or native *G. hirsutum*, in *G. tomentosum*, or in cultivated, naturalized, or native *G. barbadense*. Thus, in the unlikely event that transgene introgression from GHB811 cotton to one of these other types of cotton were to occur, the herbicide resistance traits would provide a selective advantage only when the resulting hybrids were in contact with the herbicide (i.e., in an agricultural field or treated rights of way). However, APHIS could find no reports that any of these potential recipient populations are actively controlled by herbicides. Therefore, transgene introgression from GHB811 cotton would not be expected to adversely impact recipient plants or increase their fitness or weediness any more than would gene flow from cultivated non-transgenic Upland cotton. Nor would it affect efforts to remove wild populations, as no such efforts exist. .

Based on the information presented in the petition and in relevant literature, APHIS has reached the following conclusions. The genetic modification in the GHB811 cotton is not expected to increase the potential for gene flow, hybridization and/or introgression to occur to sexually compatible taxa compared to the nontransgenic recipient or other varieties of the crop commonly grown. Gene flow, hybridization and/or introgression of genes from GHB811 to other sexually compatible relatives, including wild, weedy, feral or cultivated species in the United States and its territories is not likely to occur. Moreover, even should gene flow occur, the herbicide resistance traits are unlikely to provide a selective advantage on the resulting hybrids and additional herbicides are available to control any hybrids. Therefore, GHB811 cotton is not expected to increase the weed risk potential of other species with which it can interbreed in the United States and its territories.

H. Potential Changes to Agriculture or Cultivation Practices

APHIS assessed whether significant changes to agricultural or cultivation practices from adoption of GHB811 cotton are likely to impact plant diseases or pests or their management, including any APHIS control programs. This includes consideration of any changes in pesticide applications, tillage, irrigation, harvesting, etc. as they relate to plant pests and diseases.

IFT is a Group 27 herbicide as classified by the Weed Science Society of America (WSSA). Currently, there are no Group 27 herbicides labeled for use on cotton. As stated in Section B of this document, Bayer anticipates labeling IFT for use on HPPD-inhibitor tolerant cotton varieties developed with event GHB811 for pre-emergence (PRE) and early post-emergence (EPO) uses. The varieties Bayer expects to develop with GHB811 cotton would include three other events with phenotypes conferring glufosinate and lepidopteran resistance.

There are several herbicides with different modes of action than IFT currently labeled for pre-emergence (PRE) and post-emergence use in cotton (Table 9.2; p. 147; Bayer 2017a). The proposed timing of IFT applications would not differ from that of other herbicides used currently on cotton (Morgan et al. 2013).

HPPD-inhibitors are already used in rotational crops of cotton such as corn, sorghum and small grains (HRAC 2014). The primary crops planted after cotton are cotton (54%), corn (16%), wheat (9%), soybean (8%), sorghum (8%), and peanut (4%) (Monsanto 2012). Soybean currently has two determinations of nonregulated status with HPPD-inhibitor resistance (USDA-APHIS-BRS 2017). The use of HPPD-inhibitors have not been registered on soybean by the U.S. EPA as of early 2018, however the proposed label is currently under review (Bayer 2018). Crop rotation practices are not expected to be adversely impacted by the use of IFT on fields planted to GHB811 cotton.

Herbicides can impact pests or pathogens directly or indirectly through effects on the control of the crop or weeds associated with the crop. As noted in Section D. “Potential Plant Pest and Disease Impacts” above, field studies demonstrated that neither the herbicide resistance traits nor the herbicide treatments appear to alter the response of GHB811 cotton to abiotic stress, diseases, or arthropod pests under natural levels of these stressors, nor were pest arthropods more

abundant around GHB811 than the conventional varieties planted or the parental Coker 312 variety (Tables 8.7 and 8.8; p. 132-133; Bayer 2017a).

Other than the use of IFT to control weeds, none of the management practices currently employed for conventional cotton cultivation are expected to change if GHB811 cotton is no longer subject to the regulatory requirements of 7 CFR part 340 or the plant pest provisions of the Plant Protection Act. Bayer demonstrated that the cultivation practices needed for growing GHB811 are indistinguishable from practices used to grow conventional and glyphosate resistant cotton varieties with the exception of the application of IFT for weed control (Section 9.2; Bayer 2017a). In addition, no differences in insect or disease damage were observed in field trials with GHB811 (Tables 8.7 and 8.8; p. 132-133; Bayer 2017a).

Because agricultural and cultivation practices would not be significantly different than that of conventional cotton except for the addition of IFT to PRE and EPO herbicidal applications, no impact on plant diseases or pests or their management is likely to occur as a result of the adoption of GHB811 cotton .

I. Potential Impacts from Transfer of Genetic Information to Organisms with which GHB811 Cotton Cannot Interbreed

APHIS examined the potential for the new genetic material inserted into GHB811 cotton to be horizontally transferred without sexual reproduction to other organisms and whether such an event could lead directly or indirectly to disease, damage, injury or harm to plants, including the creation of new or more virulent pests, pathogens, or parasitic plants. The horizontal gene transfer between unrelated organisms is one of the most intensively studied fields in the biosciences since 1940, and the issue gained extra attention with the release of transgenic plants into the environment (Dröge et al. 1998). Potential risks from stable horizontal gene transfer (HGT) from genetically engineered organisms to another organism without reproduction or human intervention were recently reviewed (Keese 2008). Mechanisms of HGT include conjugation, transformation and transduction, and other diverse mechanisms of DNA and RNA uptake and recombination and rearrangement, most notably through viruses and mobile genetic elements. HGT has been a major contributor to the spread of antibiotic resistance amongst pathogenic bacteria; emergence of increased virulence in bacteria, eukaryotes and viruses; and, in the long run, to major transitions in evolution.

Potential for horizontal gene transfer to bacteria, fungi, or invertebrates

GHB11 cotton has one gene derived from bacteria. Horizontal gene transfer and expression of DNA from a plant species to bacterial, fungal or invertebrate species is unlikely to occur based on the following observations. Although there are many opportunities for plants to directly interact with fungi and bacteria (e.g. as commensals, symbionts, parasites, pathogens, decomposers, or in the guts of herbivores) and with invertebrates as plant pests, there are almost no evolutionary examples of HGT from eukaryotes to bacteria or from plants to fungi or invertebrates (Keese 2008). Examples of HGT between eukaryotes and fungi primarily involve gene acquisition or transfer by fungi to or from other distantly related fungi or bacteria (Keeling and Palmer 2008; Keese 2008) and HGT between plants and fungi is extremely rare (Richards et

al. 2009). Examples of HGT between plants and invertebrates are also extremely rare, and most examples of HGT in insects involve acquisition of genes from their pathogens or endosymbionts (Keese 2008; Zhu et al. 2011; Acuna et al. 2012).

Horizontal transfer from and expression in bacteria of the foreign DNA inserted into the nuclear genome of the GE plant is unlikely to occur. First, many genomes (or parts thereof) have been sequenced from bacteria that are closely associated with plants including *Agrobacterium* and *Rhizobium* (Wood et al. 2001; Kaneko et al. 2002). There is no evidence that these organisms contain genes derived from plants. HGT from plants to bacteria is a very low frequency event, primarily because functional and selective barriers to HGT increase with genetic distance (Keese 2008). Second, in cases where review of sequence data implied that horizontal gene transfer occurred, these events are inferred to occur on an evolutionary time scale on the order of millions of years (Brown 2003; EFSA 2009; Koonin et al. 2011). Third, transgene DNA promoters and coding sequences are optimized for plant expression, not prokaryotic bacterial expression. Thus even if horizontal gene transfer occurred, proteins corresponding to the transgenes are not likely to be produced. Fourth, both the FDA (1998) and the European Food Safety Authority (2009) have evaluated horizontal gene transfer from the use of antibiotic resistance marker genes and concluded that the likelihood of transfer of antibiotic resistance genes from plant genomes to microorganisms in the gastrointestinal tract of humans or animals, or in the environment, is very rare or remote.

Potential for horizontal gene transfer to viruses

GHB811 cotton contains sequences from the plant virus, Cassava Vein Mosaic Virus; specifically, a non-coding region used as the promoter for the *hppd* gene. APHIS also considered whether horizontal transfer of DNA from the GE plant to plant viruses was likely to occur and would lead to the creation or selection of plant viruses that are more virulent or have a broader host range. This issue has been considered before by other science review panels and government regulatory bodies (EPA 2006; Keese 2008). HGT is not unusual among plant viruses; however this is generally limited to exchange between viruses present in the same host organism in mixed infections, and most commonly involves homologous recombination, relying on sequence similarity at the point of crossover (Keese 2008). HGT from virus sequences engineered into plants has been demonstrated with infecting or challenge viruses, including both DNA viruses, e.g. geminiviruses which replicate in the nucleus (Frischmuth and Stanley 1998) and RNA viruses which typically replicate in the cytoplasm; however most have been under conditions that favor recombination to restore a defective virus (Fuchs and Gonsalves 2007; Keese 2008; Thompson and Tepfer 2010). Populations of recombinants between virus transgenes expressed in transgenic plants infected with related viruses are similar to recombinants found in mixed infections of the same viruses in nontransgenic plants, indicating that there was no novel recombination mechanism in the transgenic plants and no increased risk is expected over what is expected from mixed infections (Keese 2008; Turturo et al. 2008). Nonhomologous recombination in HGT among viruses or between virus transgenes and infecting viruses can occur, but frequently results in gene deletions which can result in nonviable viruses (Morroni et al. 2013). Depending on the particular virus and sequences involved, various hot-spots for recombination have been found in both coding and noncoding regions, and strategies implemented in design of transgenes to avoid recombination have been suggested. No recombinant or undesirable viruses with new properties have been detected for over at least 8-10

years in field tests or during commercial growth of deregulated virus resistant plum, squash, or papaya engineered with genes from viruses that have been deregulated in the U.S. (Fuchs and Gonsalves 2007).

Potential for horizontal gene transfer to parasitic plants

Evidence for HGT from plants to other plants is limited to two specific scenarios: (1) exchange of genes between a parasitic plant and its host; and (2) exchange of genes between cells of two plants living in close proximity, such as in a graft junction. In both cases, this type of HGT requires physical contacts between the two plants. Most cases of HGT in plants involve transfer of mitochondrial genomes, which are primarily maternally inherited in plants (Barr et al. 2005), to other mitochondria genomes, and mostly involve parasitic plants and their hosts (Richardson and Palmer 2007). Recently, through a comparative genomics analysis implicated HGT for the incorporation of a specific genetic sequence in the parasitic plant purple witchweed (*Striga hermonthica*) from its monocot host plant, (Yoshida et al. 2010). According to this study, the incorporation of the specific genetic sequence (with an unknown function) occurred between sorghum and purple witchweed. However, this HGT occurred before speciation of purple witchweed and related cowpea witchweed (*S. gesnerioides*) from their common ancestor. Furthermore, *S. hermonthica* is not found in the U.S. and *S. asiatica*, another related parasite of cereal crops, is only present in North Carolina and South Carolina (USDA-NRCS 2013). More recent studies demonstrated that in a few parasitic species of the Rafflesiaceae family, out of several genetic sequences examined, about 2.1% of nuclear (Xi et al. 2012) and 24 –41% of mitochondrial (Xi et al. 2013) gene transcripts appeared to be acquired from their obligate host species. However, all the above-mentioned instances of HGT between parasitic plants and their hosts were reported to be of ancient origins, on an evolutionary time scale spanning thousands to millions of years ago. Furthermore in GHB811 cotton, the DNA sequences were inserted into the nuclear genome, not the mitochondrial genome (Bayer 2017a).

If GHB811 cotton becomes infected by a parasitic plant or is naturally grafted to another plant, there is a very low probability that HGT could result in the other plant acquiring DNA from GHB811 cotton. However, in both scenarios this newly introduced DNA would likely reside in somatic cells, and with little chance of reaching the germ cells, this introduced DNA could not persist in subsequent generations unless the recipient plant reproduced asexually from the affected cells.

Based on the above analysis APHIS therefore concludes that HGT of the new genetic material inserted into GHB811 cotton to other organisms is highly unlikely, and is not expected to lead directly or indirectly to disease, damage, injury or harm to plants, including the creation of new or more virulent pests, pathogens, or parasitic plants.

J. Conclusion

APHIS has reviewed the information submitted in the petition, supporting documents, and other relevant information to assess the plant pest risk of GHB811 cotton compared to the unmodified variety from which it was derived. APHIS concludes that GHB811 cotton is unlikely to pose a plant pest risk based on the following findings.

- No plant pest risk was identified from the transformation process or the insertion of new genetic material in GHB811 cotton: the *Agrobacterium* transformation vector was eliminated from the transformed material using antibiotics, and the plant pest sequences inserted do not cause disease, create an infectious agent, or otherwise confer any plant pest characteristics to GHB811 cotton.
- No increase in plant pest risk was identified in GHB811 cotton from expression of the inserted genetic material, the 2mEPSPS or HPPD W336 proteins, or changes in metabolism or composition. GHB811 cotton seed can be considered compositionally and nutritionally equivalent to seed derived from conventional cotton and the mode of action and specificity of the introduced proteins raise no plant pest concerns.
- Disease and pest incidence and/or damage were not observed to be significantly increased or atypical in GHB811 cotton compared to the nontransgenic counterpart or other comparators in field trials conducted in growing regions representative of where GHB811 cotton is expected to be grown. Observed agronomic traits also did not reveal any biologically relevant differences that would indirectly indicate that the GE crop event is more susceptible to pests or diseases**.** Therefore no plant pest effects are expected on these or other agricultural products and no impacts are expected to APHIS pest control programs.
- Exposure to and/or consumption of GHB811 cotton are unlikely to have any adverse impacts on organisms beneficial to agriculture based on the analysis of compositional, phenotypic and agronomic data and food and feed safety studies.
- GHB811 cotton is no more likely to become a weed than conventional varieties of the crop based on its observed agronomic characteristics, weediness potential of the crop and current management practices available to control GHB811 cotton as a weed. Volunteers and feral populations of the herbicide resistant GE crop event can be managed using a variety of currently available methods and alternative herbicides**.**
- GHB811 cotton is not expected to increase the weed risk potential of other species with which it can interbreed in the U.S. or its territories. Gene flow, hybridization and/or introgression of inserted genes from the GE crop event to other sexually compatible relatives with which it can interbreed is not likely to occur. These compatible relatives are not considered weedy or invasive. The new phenotypes conferred by genetic engineering are not likely to increase the weediness of these compatible relatives or affect the current ability to control these relatives.
- Significant changes to agricultural or cultivation practices (e.g. pesticide applications, tillage, irrigation, harvesting, etc.) from adoption of GHB811 cotton are not likely to increase plant diseases or pests or compromise their management.
- Horizontal gene transfer of the new genetic material inserted into GHB811 cotton to other organisms is highly unlikely, and is not expected to lead directly or indirectly to disease, damage, injury or harm to plants, including the creation of new or more virulent pests, pathogens, or parasitic plants.

K. References

51 FR 23302. 1986. *Coordinated Framework for Regulation of Biotechnology*. Retrieved from https://www.govinfo.gov/app/details/FR-1986-06-26.

57 FR 22984. 1992. *Statement of Policy: Foods Derived from New Plant Varieties*. Retrieved from https://www.govinfo.gov/app/details/FR-1992-05-29.

Acuna R, Padilla BE, Florez-Ramos CP, Rubio JD, Herrera JC, Benavides P, Lee SJ, Yeats TH, Egan AN, Doyle JJ, and Rose JK. 2012. *Adaptive horizontal transfer of a bacterial gene to an invasive insect pest of coffee*. Proc Nat Acad Sci 109, pp. 4197-4202. Retrieved from http://www.pnas.org/content/early/2012/02/17/1121190109.abstract

Agricultural Marketing Resource Center. 2012. *Cotton Profile*. Agricultural Marketing Resource Center. Retrieved from http://www.agmrc.org/commodities-products/fiber/cotton/ Last accessed 08/03/2017.

Amrhein N, Deus B, Gehrke P, and Steinrucken HC. 1980. *The Site of the Inhibition of the Shikimate Pathway by Glyphosate: II. INTERFERENCE OF GLYPHOSATE WITH CHORISMATE FORMATION IN VIVO AND IN VITRO*. Plant Physiol 66, pp. 830-834. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/16661535

AOSCA. 2012. *Seed Certification Handbook*. Moline, IL. Association of Official Seed Certifying Agencies, pp. 166.

Baker HB. 1965. Characteristics and Modes of Origin of Weeds. In: *The Genetics of Colonizing Speciess* (New York & London: Academic Press, eds. HG Baker; GL Stebbin), pp. 147-172.

Barr CM, Neiman M, and Taylor DR. 2005. *Inheritance and recombination of mitochondrial genomes in plants, fungi and animals*. New Phytologist 168, pp. 39-50. Retrieved from http://onlinelibrary.wiley.com/doi/10.1111/j.1469-8137.2005.01492.x/abstract

Baughman T. 2011. *AgriLife Extension specialist: Cotton planting should be delayed in times of drought*. today.agrilife.org. Retrieved from http://today.agrilife.org/2011/05/03/agrilife-extension-specialist-cotton-planting-shouldbe-delayed-in-times-of-drought/ Last accessed 12/11/2017.

Bayer CropScience. 2006. *Determination of Non-regulated Status for Glyphosate-Tolerant (GlyTol™) Cotton, Gossypium hirsutum, event GHB614*. Submitted by Alejandra L. Scott Ph.D., Regulatory Affairs Manager. Bayer CropScience. Research Triangle Park, NC. Retrieved from https://www.aphis.usda.gov/aphis/ourfocus/biotechnology/permits-notifications-petitions/petitions/petition-status Last accessed 09/08/2017.

Bayer CropScience. 2017a. *Petition for a Determination of Nonregulated Status for Herbicide Tolerance Cotton Transformation Event GHB811*. Submitted by M. Weeks, M.Sc. Bayer CropScience. Research Triangle Park, NC. Last accessed Original petition recieved May 16, 2017. Final amended petition recieved July 21, 2017.

Bayer. 2017b. *Personal Communication: Confirmation of Disarmed A. tumefaciens. Received Dec. 19, 2017.*

Bayer. 2018. *Bayer CropScience: Traits*. Bayer CropScience. Retrieved from https://www.cropscience.bayer.us/products/traits Last accessed 01/09/2018.

Boudec P, Rodgers M, Dumas F, Sailland A, and Bourdon H. 2001. *Mutated Hydroxyphenylpyruvate Dioxygenase, DNA Sequence and Isolation of Plants which Contain Such a Gene and Which are Tolerant to Herbicides:* United States Patent: 6,245,968 B1. United States.

Brown JR. 2003. *Ancient horizontal gene transfer*. Nature Reviews Genetics 4, pp. 121-132. Retrieved from http://www.nature.com/nrg/journal/v4/n2/full/nrg1000.html

Brownlee JM, Johnson-Winters K, Harrison DHT, and Moran GR. 2004. *Structure of the Ferrous Form of (4-Hydroxyphenyl)pyruvate Dioxygenase from Streptomyces avermitilis in Complex with the Therapeutic Herbicide, NTBC*. Biochemistry 43, pp. 6370-6377. Retrieved from http://dx.doi.org/10.1021/bi049317s

Brubaker CL, Koontz JA, and Wendel JF. 1993. *Bidirectional Cytoplasmic and Nuclear Introgression in the New World Cottons, Gossypium barbadense and G. hirsutum (Malvaceae)*. American Journal of Botany 80, pp. 1203-1208. Retrieved from http://www.jstor.org/stable/2445549

Charles G, Roberts G, Kerlin S, and Hickman M. 2013. *Controlling Volunteer Cotton-a guide to integrated weed management in cotton*. Australia. Australian Government, Cotton Research and Development Corporation. Retrieved from http://www.insidecotton.com/xmlui/handle/1/995 Last accessed 12/11/2017.

Chaubet N, Clement B, and Gigot C. 1992. *Genes Encoding a Histone H3.3-like Variant in Arabidopsis Contain Intervening Sequences*. Journal of Molecular Biology 225, pp. 569-574.

Chauboute M, Chaubet N, Philipps G, Ehling M, and Gigot C. 1987. *Genomic Organization and Nucleotide Sequences of Two Histone H3 and Two Histone H4 genes of Arabidopsis thaliana*. Plant Molecular Biology 8, pp. 179-191.

Coile N and Garland M. 2003. *Notes on Florida's Endangered and Threatened Plants* (38, pp 127). Gainesville, FL. Florida Deptartment of Agriculture and Consumer Services. Last accessed 09/08/2017.

Crocket LJ. 1977. Wildly successful plants. In: *A handbook of North American weeds* (New York: MacMillan), pp. 268.

DeJoode DR and Wendel JF. 1992. *Genetic Diversity and Origin of the Hawaiian Islands Cotton, Gossypium tomentosum*. American Journal of Botany 79, pp. 1311-1319. Retrieved from http://www.jstor.org/stable/2445059?origin=JSTOR-pdf&seq=1#page_scan_tab_contents

Dröge M, Pühler A, and Selbitschka W. 1998. *Horizontal gene transfer as a biosafety issue: A natural phenomenon of public concern*. Journal of Biotechnology 64, pp. 75–90. Retrieved from http://www.sciencedirect.com/science/article/pii/S0168165698001059

EFSA. 2009. *Statement of EFSA on the consolidated presentation of opinions on the use of antibiotic resistance genes as marker genes in genetically modified plants*. European Food Safety Authority. Retrieved from http://www.efsa.europa.eu/en/efsajournal/pub/1108.htm Last accessed 09/08/2017.

Ellstrand NC, Prentice HC, and Hancock JF. 1999. *Gene flow and introgression from domesticated plants into their wild relatives*. Annual Review of Ecology and Systematics 30, pp. 539-563. Retrieved from http://www.annualreviews.org/doi/abs/10.1146%2Fannurev.ecolsys.30.1.539

EPA. 2006. *Plant Incorporated Protectants Based on Virus Coat Protein Genes: Science Issues Asosciated with the Proposed Rule*. Washington, DC. Environmental Protection Agency. Retrieved from http://www.epa.gov/scipoly/sap/meetings/2005/december/minutes1205.pdf Last accessed 09/08/2017.

Fang H, Zhou H, Sanogo S, Lipka AE, Fang DD, Percy RG, Hughs SE, Jones DC, Gore MA, and Zhang J. 2013. *Quantitative trait locus analysis of Verticillium wilt resistance in an introgressed recombinant inbred population of Upland cotton*. Molecular Breeding 33, pp. 709-720.

Fannin B. 2010. *Producers battling volunteer cotton in corn, sorghum fields*. College Station, TX. Texas A&M University. Retrieved from http://today.agrilife.org/2010/12/24/volunteer-cotton/ Last accessed 12/11/2017.

FDA. 1998. *Guidance for Industry: Use of Antibiotic Resistance Marker Genes in Transgenic Plants*. Washington, DC. Food and Drug Administration. Retrieved from http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/Biotechnology/ucm096135.htm Last accessed 09/08/2017.

FDA. 2006. *Guidance for Industry: Recommendations for the Early Food Safety Evaluation of New Non-Pesticidal Proteins Produced by New Plant Varieties Intended for Food Use*. Washington, DC. Food and Drug Administration. Retrieved from http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/Biotechnology/ucm096156.htm Last accessed 09/21/2017.

FDA. 2017. *Biotechnology Consultations on Food from GE Plant Varieties* Washington, DC. Food and Drug Administration. Retrieved from https://www.accessdata.fda.gov/scripts/fdcc/?set=Biocon Last accessed 09/08/2017.

Fernandez-Cornejo J., Nehring R, Osteen C, Wechsler S, Martin A, and Vialou A. 2014. *Pesticide Use in U.S. Agriculture: 21 Selected Crops, 1960-2008* (EIB-124). U.S. Department of Agriculture, Economic Research Service. Retrieved from https://www.ers.usda.gov/publications/pub-details/?pubid=43855 Last accessed 09/08/2017.

Frischmuth T and Stanley J. 1998. *Recombination between viral DNA and the transgenic coat protein gene of African cassava mosaic geminivirus*. Journal of General Virology 79, pp. 1265-1271. Retrieved from http://vir.sgmjournals.org/content/79/5/1265

Fritze IM, Linden L, Freigang J, Auerbach G, Huber R, and Steinbacher S. 2004. *The Crystal Structures of Zea mays and Arabidopsis 4-Hydroxyphenylpyruvate Dioxygenase*. Plant Physiology 134, pp. 1388-1400. Retrieved from http://www.plantphysiol.org/content/134/4/1388.abstract

Fromme D, Grichar J, Morgan G, and Bean B. 2011. *Control of Volunteer Cotton in Corn* (Conference Presentation). Texas Boll Weevil Eradication Foundation, Technical Advisory Committee. February 15, 2011, McAllen, TX.

Fryxell PA. 1979. *The Natural History of the Cotton Tribe (Malvaceae, Tribe Gossypieae)*. College Station, TX.: Texas A&M University Press.

Fryxell PA. 1984. Taxonomy and Germplasm Resources. In: *Cotton, Agronomy Monograph 24, 1984* (Madison, WI: ASA, CSA, SSA, eds. CW Smith and Cothren JT), pp. 27-57. Retrieved from https://dl.sciencesocieties.org/publications/books/tocs/agronomymonogra/cotton Last accessed 09/08/2017.

Fuchs M and Gonsalves D. 2007. *Safety of virus-resistant transgenic plants two decades after their introduction: lessons from realistic field risk assessment studies*. Annual Review of Phytopathology 45, pp. 173-202. Retrieved from http://www.annualreviews.org/doi/full/10.1146/annurev.phyto.45.062806.094434?url_ver=Z39.88-2003

Funke T, Yang Y, Han H, Healy-Fried M, Olesen S, Becker A, and Schönbrunn E. 2009. *Structural Basis of Glyphosate Resistance Resulting from the Double Mutation Thr97 → Ile and Pro101 → Ser in 5-Enolpyruvylshikimate-3-phosphate Synthase from Escherichia coli*. Journal of Biological Chemistry 284, pp. 9854-9860. Retrieved from http://www.jbc.org/content/284/15/9854.abstract

Grant V. 1981. Plant Speciation, 2nd Edition (New York, NY: Columbia University Press), pp. 563.

Green JM and Jones MD. 1953. *Isolation of cotton for seed increase*. Agronomy Journal 45, pp. 366-368.

Harrison LA, Bailey MR, Naylor MW, Ream JE, Hammond BG, Nida DL, Burnette BL, Nickson TE, Mitsky TA, Taylor ML, Fuchs RL, and Padgette SR. 1996. *The expressed protein in glyphosate-tolerant soybean, 5-enolpyruvylshikimate-3-phosphate synthase from Agrobacterium sp. strain CP4, is rapidly digested in vitro and is not toxic to acutely gavaged mice*. J Nutr 126, pp. 728-740. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/8598558

Hegde SG, Nason JD, Clegg JM, Ellstrand NC, and Soltis P. 2006. *The Evolution of California's Wild Radish Has Resulted in the Extinction of Its Progenitors*. Evolution 60, pp. 1187-1197. Retrieved from https://doi.org/10.1554/05-634.1

Hellens R and Mullineaux P. 2000. *A guide to Agrobacterium binary Ti vectors*. Trends in Plant Science 5, pp. 447-451.

Hoess R and Abremski K. 1985. *Mechanism of Strand Cleavage and Exchange in the Cre-lox Site-specific Recombination System*. Journal of Molecular Biology 181, pp. 351-362.

Holm LG, Pancho JV, Herberger JP, and Plucknett DL. 1979. *A Geographical Atlas of World Weeds*. New York, NY: John Wiley and Sons.

HRAC. 2014. *HPPD-inhibitor Resistance Stewardship: The Perspective of the HRAC HPPD-inhibitor Working Group*. Herbicide Resistance Action Committee. Retrieved from http://hracglobal.com/files/HPPD-inhibitor-Fact-Sheet.pdf Last accessed 01/09/2018.

ILSI. 2016a. *A Review of the Food and Feed Safety of the EPSPS Protein*. Washington, DC. ILSI Research Foundation, DOI 10.13140/RG.2.2.15073.10081. Last accessed 01/12/2018.

ILSI. 2016b. ILSI CCDB version 6: *International Life Sciences Institute Crop Compositional Database version 6*. Retrieved from https://www.cropcomposition.org/query/index.html Last accessed 07/06/2017.

Jiang C, Chee PW, Draye X, Morrell PL, Smith CW, and Paterson AH. 2000. *Multilocus interactions restrict gene introgression in interspecific populations of polyploid Gossypium (cotton)*. Evolution 54, pp. 798-814.

Johnson BC, Young BG, and Matthews JL. 2002. *Effect of Postemergence Application Rate and Timing of Mesotrione on Corn (Zea mays) Response and Weed Control*. Weed Technology 16, pp. 414-420. Retrieved from https://doi.org/10.1614/0890-037X(2002)016[0414:EOPARA]2.0.CO;2

Kaneko T, Nakamura Y, Sato S, Minamisawa K, Uchiumi T, Sasamoto S, Watanabe A, Idesawa K, Iriguchi M, Kawashima K, Kohara M, Matsumoto M, Shimpo S, Tsuruoka H, Wada T, Yamada M, and Tabata S. 2002. *Complete Genomic Sequence of Nitrogen-fixing Symbiotic Bacterium Bradyrhizobium japonicum USDA110*. DNA Research 9, pp. 189–197. Retrieved from http://dnaresearch.oxfordjournals.org/content/9/6/189.long

Keeler K. 1989. *Can Genetically Engineered Crops Become Weeds?* Biotechnology 7, pp. 1134-1139.

Keeling JW, Reed JD, Petty DM, and Brown BA. 2009. *Contro of Volunteer Roundup Ready Flex Cotton with Postemergence Herbicides* (Conference Presentation). 2009 Beltwide Cotton Conferences. January 5-8, 2009, San Antonio, TX. Retrieved from http://agrilife.org/lubbock/files/2011/10/ControlofVolunteerRRCottonwithPostemergenceHerbicides.pdf Last accessed 12/11/2017.

Keeling PJ and Palmer JD. 2008. *Horizontal gene transfer in eukaryotic evolution*. Nature Reviews Genetics 9, pp. 605-618. Retrieved from http://www.nature.com/nrg/journal/v9/n8/full/nrg2386.html

Keese P. 2008. *Risks from GMOs due to horizontal gene transfer*. Environmental Biosafety Research 7, pp. 123-149. Retrieved from http://www.ebr-journal.org/action/displayAbstract?fromPage=online&aid=8208895

Khoury CK, Greene S, Wiersema J, Maxted N, Jarvis A, and Struik PC. 2013. *An Inventory of Crop Wild Relatives of the United States*. Crop Science 53, pp. 1496-1508. Retrieved from https://www.crops.org/publications/cs/abstracts/53/4/1496

Koonin EV, Makarova KS, and Aravind L. 2011. *Horizonal Gene Transfer in Prokaryotes: Quantification and Classification*. Annual Review of Microbiology 55, pp. 709-742. Retrieved from http://www.annualreviews.org/doi/full/10.1146/annurev.micro.55.1.709?url_ver=Z39.88-2003

Lebrun M, Leroux B, and Sailland A. 1996. *Chimeric Gene for the Transformation of Plants:* US Patent: 5,510,471. US.

Lebrun M, Sailland A, and Freyssinet G. 1997. *Mutated 5-enol pyruvylshikimate-3-phosphate synthase, Gene Coding for Said Protein and Transformed Plants Containing Said Gene:* FR Patent: WO 97/04103. Lyons: FR.

Ledbetter K. 2011. *Cotton planting should be delayed in times of drought* (http://today.agrilife.org/2011/05/03/agrilife-extension-specialist-cotton-planting-should-be-delayed-in-times-of-drought/). College Station, TX. Texas A&M University. Last accessed 12/11/2017.

Lee J and Fang D. 2015. Cotton as a World Crop: Origin, History, and Current Status. In: *Cotton, 2nd edition* (Madison, WI: eds. RJ Kohel and CF Lewis, ASA, CSSA, and SSSA), pp. 1-25.

Lee JA. 1984. Cotton as a World Crop. In: *Cotton* (Madison, WI: eds. RJ Kohel and CF Lewis, ASA, CSSA, and SSSA), pp. 1-25. Retrieved from http://dx.doi.org/10.2134/agronmonogr24.c1

Llewellyn D, Tyson C, Constable G, Duggan B, Beale S, and Steel P. 2007. *Containment of regulated genetically modified cotton in the field*. Agriculture, Ecosystems & Environment 121, pp. 419-429. Retrieved from http://www.sciencedirect.com/science/article/pii/S0167880906004543

McGregor SE. 1976. *Insect Pollination Of Cultivated Crop Plants*. USA: United States Deparment of Agriculture Retrieved from https://www.amazon.com/Insect-Pollination-Cultivated-Crop-Plants/dp/B002CIQFZ2

Mississippi State University. 2016. *Insect Cotton Losses*. Retrieved from http://www.biochemistry.msstate.edu/resources/croplosses/2016loss.asp Last accessed 09/07/2017.

Monsanto Company. 1995. *Petition for the Determination of Non-Regulated Status: Cotton with Roundup Ready Gene, lines 1445 and 1698*. Submitted by F.S. Serdy. Monsanto

Company. Chesterfield, MO. Retrieved from https://www.aphis.usda.gov/aphis/ourfocus/biotechnology/permits-notifications-petitions/petitions/petition-status Last accessed 12/19/2017.

Monsanto Company. 2004. *Petition for the Determination of Non-Regulated Status for Roundup Ready Flex Cotton MON 88913*. Submitted by J.A. Burns, Ph.D. Monsanto Company. St. Louis, MO. Retrieved from https://www.aphis.usda.gov/aphis/ourfocus/biotechnology/permits-notifications-petitions/petitions/petition-status Last accessed 12/19/2017.

Monsanto Company. 2012. *Petition for the Determination of Nonregulated Status for Dicamba and Glufosinate-Tolerant Cotton MON 88701*. Submitted by M. Malven, M.S. Monsanto Company. St. Louis, MO. Retrieved from https://www.aphis.usda.gov/aphis/ourfocus/biotechnology/permits-notifications-petitions/petitions/petition-status Last accessed 01/09/2018.

Morgan GD, Baumann PA, and Dotray PA. 2013. *Weed Management in Texas Cotton* (SCS-2013-09). College Station, TX. Texas A&M University. Retrieved from http://publications.tamu.edu/WEEDS_HERBICIDES/WeedControl_Cotton%20revision%202012%20Final.pdf Last accessed 12/11/2017.

Morgan GD, Keeling JW, Baumann PA, and Dotray PA. 2011a. *Managing Volunteer Cotton in Cotton* (Extension SCS-2011-05). College Station, TX. Texas A&M University. Retrieved from http://cotton.tamu.edu/Weeds/Managing%20Volunteer%20Cotton%20in%20Cotton%20-%20Morgan%20(2).pdf Last accessed 12/11/2017.

Morgan GD, Fromme DA, Baumann PA, Grichar J, Bean B, Matocha ME, and Mott DA. 2011b. *Managing Volunteer Cotton in Grain Crops* (Extension SCS-2011-04). College Station, TX. Texas A&M University. Retrieved from http://publications.tamu.edu/COTTON/PUB_cotton_Managing%20Volunteer%20Cotton%20in%20Grain%20Crops.pdf Last accessed 12/11/2017.

Morroni M, Jacquemond M, and Tepfer M. 2013. *Deep sequencing of recombinant virus populations in transgenic and nontransgenic plants infected with Cucumber mosaic virus*. Molecular Plant-Microbe Interactions 26, pp. 801-811. Retrieved from http://apsjournals.apsnet.org/doi/abs/10.1094/MPMI-02-13-0057-R?url_ver=Z39.88-2003

Muenscher WC. 1980. *Weeds*. New York and London: Cornell University Press.

Münster P and Wieczorek AM. 2007. *Potential gene flow from agricultural crops to native plant relatives in the Hawaiian Islands*. Agriculture, Ecosystems & Environment 119, pp. 1-10.

National Cotton Council. 2016. Disease Database: *Pest Management*. Retrieved from http://www.cotton.org/tech/pest/ Last accessed 09/07/2017.

National Cotton Council. 2018. *Pink Bollworm Eradication Program*. National Cotton Council. Retrieved from http://www.cotton.org/tech/pest/bollworm/ Last accessed 01/09/2018.

OECD. 1993. In: *Traditional Crop Breeding Practices: An Historical Review to Serve as a Baseline for Assessing the Role of Modern Biotechnology.* (Paris, France: Organisation for Economic Co-operation and Development), pp. 235. Retrieved from https://www.cabdirect.org/cabdirect/abstract/19941609184 Last accessed 09/08/2017.

OECD. 2008. *Consensus Document on the Biology of Cotton (Gossypium spp.)* (No. 45). Organisation for Economic Co-operation and Development. Retrieved from http://www.oecd.org/science/biotrack/46815918.pdf Last accessed 09/08/2017.

OGTR. 2008. *The Biology of Gossypium hirsutum L. and Gossypium barbadense L. (cotton).* Australia. Office of the Gene Technology Regulator Retrieved from http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/biologycotton-toc Last accessed 09/08/2017.

Percy RG and Wendel JF. 1990. *Allozyme evidence for the origin and diversification of Gossypium barbadense L.* Theoretical and Applied Genetics 79, pp. 529-542. Retrieved from http://link.springer.com/article/10.1007/BF00226164

Pleasants JM and Wendel JF. 2005. *Assessment of potential for gene flow between transgenic cotton and the endemic Hawaiian cotton* (83032901). Ames, Iowa. Iowa State University Retrieved from http://www.public.iastate.edu/~jpleasan/publications.html Last accessed 09/08/2017.

Pleasants JM and Wendel JF. 2010. *Reproductive and Pollination Biology of the Endemic Hawaiian Cotton, Gossypium tomentosum (Malvaceae).* Pacific Science 64, pp. 45-55. Retrieved from http://www.bioone.org/doi/full/10.2984/64.1.045

Richards TA, Soanes DM, Foster PG, Leonard G, Thornton CR, and Talbot NJ. 2009. *Phylogenomic analysis demonstrates a pattern of rare and ancient horizontal gene transfer between plants and fungi*. Plant Cell 21, pp. 1897-1911. Retrieved from http://www.plantcell.org/content/21/7/1897.long

Richardson AO and Palmer JD. 2007. *Horizontal gene transfer in plants*. Journal of Experimental Botany 58, pp. 1-9. Retrieved from http://jxb.oxfordjournals.org/content/58/1/1.long

Rieseberg LH and Wendel JF. 1993. *Introgression and its consequences in plants*.

Rippert P, Scimemi C, Dubald M, and Matringe M. 2004. *Engineering Plant Shikimate Pathway for Production of Tocotrienol and Improving Herbicide Resistance*. Plant Physiology 134, pp. 92-100. Retrieved from http://www.plantphysiol.org/content/plantphysiol/134/1/92.full.pdf

Roberts G, Kerlin S, and Hickman M. 2002. *Controlling volunteer cotton: A Guide for Integrated Management of Weeds in Cotton*. Narrabri, New South Wales. Australian Cotton Research Institute. Retrieved from http://www.cottoncrc.org.au/industry/Publications/Weeds/WEEDpak/F1_Farm_Hygiene Last accessed 12/11/2017.

Rost TL. 1998. *Cotton Anatomy Fruit Seed Fibers*. University of Californa, Davis. Retrieved from http://www-plb.ucdavis.edu/labs/rost/cotton/reproduction/frfiber.html Last accessed 09/03/2017.

Saha S, Raska DA, and Stelly DM. 2006. *Breeding and Genetics Upland Cotton (Gossypium hirsutum L.) x Hawaiian Cotton (G. tomentosum Nutt. ex Seem.) F_1 Hybrid Hypoaneuploid Chromosome Substitution Series*. Journal of Cotton Science 10, pp. 263-272. Retrieved from https://www.researchgate.net/publication/238784418_BREEDING_AND_GENETICS_Upland_Cotton_Gossypium_hirsutum_L_x_Hawaiian_Cotton_G_tomentosum_Nutt_ex_Seem_F1_Hybrid_Hypoaneuploid_Chromosome_Substitution_Series

Saini RK and Keum Y. 2016. *Tocopherols and tocotrienols in plants and their products: A review on methods of extraction, chromatographic separation, and detection*. Food Research International 82, pp. 59-70. Retrieved from http://www.sciencedirect.com/science/article/pii/S0963996916300278

Soltis DE, Soltis PS, and Rieseberg LH. 1993. *Molecular Data and the Dynamic Nature of Polyploidy*. Critical Reviews in Plant Sciences 12, pp. 243-273. Retrieved from http://www.tandfonline.com/doi/abs/10.1080/07352689309701903#.UhekuRtwq-w

Stace CA. 1987. *Hybridization and the plant species*.

Steinrucken HC and Amrhein N. 1980. *The herbicide glyphosate is a potent inhibitor of 5-enolpyruvyl-shikimic acid-3-phosphate synthase*. Biochem Biophys Res Commun 94, pp. 1207-1212. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/7396959

Syngenta Seeds, Inc., Bayer CropScience. 2012. *Petition for Determination of Nonregulated Status for Herbicide-tolerant Event SYHT0H2 Soybean*. Submitted by Dennis P. Ward and M. Weeks. Syngenta Seeds, Inc., Bayer CropScience. Research Triangle Park, NC. Retrieved from https://www.aphis.usda.gov/aphis/ourfocus/biotechnology/permits-notifications-petitions/petitions/petition-status Last accessed 12/01/2017.

Thompson A and Steckel L. 2008. *Controlling Volunteer Cotton in Soybeans* (Extension W168). Knoxville, TN. University of Tennessee. Retrieved from https://extension.tennessee.edu/publications/Documents/W168.pdf Last accessed 12/11/2017.

Thompson JR and Tepfer M. 2010. *Assessment of the Benefits and Risks for Engineered Virus Resistance*. Advances in Virus Research 76, pp. 33-56. Retrieved from http://www.sciencedirect.com/science/article/pii/S0065352710760024

Tsegaye Y, Shintani DK, and DellaPenna D. 2002. *Overexpression of the enzyme p-hydroxyphenolpyruvate dioxygenase in Arabidopsis and its relation to tocopherol biosynthesis*. Plant Physiol. Biochem 40, pp. 913–920.

Turturo C, Friscina A, Gaubert S, Jacquemond M, Thompson JR, and Tepfer M. 2008. *Evaluation of potential risks associated with recombination in transgenic plants expressing viral sequences*. The Journal of General Virology 89, pp. 327-335. Retrieved from http://vir.sgmjournals.org/content/89/1/327.long

Ujiie A, Yamada T, Fujimoto K, Endo Y, and Kitamura K. 2005. *Identification of soybean varieties with high α-tocopherol content*. Breeding Science 55, pp. 123-125.

Umbeck PF, Barton KA, Nordheim EV, McCarthy JC, Parrott WL, and Jenkins JN. 1991. *Degree of Pollen Dispersal by Insects from a Field Test of Genetically Engineered Cotton*. Journal of Economic Entomology 84, pp. 1943-1950. Retrieved from http://jee.oxfordjournals.org/content/84/6/1943.abstract

USDA-APHIS-BRS. 2017. USDA-BRS Petition Status: *Petitions for the Determination of Nonregulated Status*. Retrieved from https://www.aphis.usda.gov/aphis/ourfocus/biotechnology/permits-notifications-petitions/petitions/petition-status Last accessed 09/08/2017.

USDA-ERS. 2017a. *U.S. Cotton Production Trends 2016*. Washington, DC. Retrieved from https://www.ers.usda.gov/topics/crops/cotton-wool/background/ Last accessed 09/08/2017.

USDA-ERS. 2017b. *Genetically engineered varieties of corn, upland cotton, and soybeans, by State and for the United States, 2000-17*. Washington, DC. United States Department of Agriculture-Economic Research Service. Retrieved from http://usda.mannlib.cornell.edu/MannUsda/viewDocumentInfo.do?documentID=1000 Last accessed 12/21/2017.

USDA-FS. 2017. *Desert Cotton*. Washington, DC. Retrieved from https://www.fs.fed.us/wildflowers/beauty/Sky_Islands/plants/Gossypium_thurberi/index.shtml Last accessed 09/08/2017.
USDA-NASS. 2012. USDA-NASS 2012 Census Data: *2012 Census Map of Pima Cotton Harvested*. Retrieved from https://agcensus.usda.gov/Publications/2012/Online_Resources/Ag_Atlas_Maps/Crops_and_Plants/Field_Crops_Harvested/12-M186-RGBDot1-largetext.pdf Last accessed August 02, 2017.
USDA-NASS. 2015. *2012 Census of Agriculture Highlights: Cotton and Cotton Seed Production*. Washington, DC. Retrieved from https://www.agcensus.usda.gov/Publications/2012/Online_Resources/Highlights/Cotton/Highlights_Cotton.pdf Last accessed 09/08/2017.
USDA-NASS. 2016. *Quik Stats Charts and Maps: 2016 Cotton Production by County in the U.S.* Washington, DC. United States Department of Agriculture. Retrieved from https://www.nass.usda.gov/Charts_and_Maps/graphics/CTU-PR-RGBChor.pdf Last accessed 12/20/2017.
USDA-NASS. 2017a. NASS Quick Stats: *Cotton Acreages Quick Stats*. Retrieved from https://www.nass.usda.gov/Statistics_by_Subject/index.php?sector=CROPS Last accessed 09/02/2017.
USDA-NASS. 2017b. *ArcGis Map of Florida Wild Upland Cotton and Commerical Upland Cotton Production*. Washington, DC. Last accessed 09/08/2017.
USDA-NRCS. 2013. *Striga asiatica (L.) Kuntze, Asiatic witchweed*. Retrieved from http://plants.usda.gov/core/profile?symbol=STAS2 Last accessed 09/21/2013.
USDA-NRCS. 2014. Plants Database: *Native, Invasive, and Other Plant-Related Definitions*. Retrieved from http://plants.usda.gov Last accessed 09/07/2017.
USDA-NRCS. 2017. Plants Database: *Introduced and Invasive Plants*. Retrieved from https://plants.usda.gov/core/profile?symbol=GOHI Last accessed 09/08/2017.
USDA-PPQ. 2014. *2014 Boll Weevil Eradication Update*. Washington, DC. Retrieved from https://www.aphis.usda.gov/plant_health/plant_pest_info/cotton_pests/downloads/bwe-map.pdf Last accessed 09/08/2017.
USDA-PPQ. 2017. *Cotton Pest Programs*. USDA-PPQ. Retrieved from https://www.aphis.usda.gov/aphis/ourfocus/planthealth/plant-pest-and-disease-programs/pests-and-diseases/cotton-pests/ct_cotton_pests_home Last accessed 09/04/2017.
Van Deynze AE, Sundstrom FJ, and Bradford KJ. 2005. *Pollen-Mediated Gene Flow in California Cotton Depends on Pollinator Activity*. Crop Science 45, pp. 1565-1570.
Van Deynze AE, Hutmacher RB, and Bradford KJ. 2011. *Gene Flow between L. and L. is Asymmetric*. Crop Science 51, pp. 298-305.
Verdaguer B, de Kochko A, Beachy RN, and Fauquet C. 1996. *Isolation and Expression in Transgenic Tobacco and Rice Plant, of the Cassava Vein Mosaic Virus (CVMV) promoter*. Plant Molecular Biology 31, pp. 1129-1139.
Wagner WL, Herbst DR, and Sohmer SH. 1990. *Manual of the flowering plants of Hawai'i*. Bishop Museum Special Publication 53. Retrieved from http://www.uhpress.hawaii.edu/p-544-9780824821661.aspx Last accessed 09/08/2017.
Wagner WL, Herbst DR, Khan N, and Flynn T. 2012. *Hawaiian vascular plant updates: a supplement to the Manual of the Flowering Plants of Hawai'i and Hawai'i's ferns and*

fern allies, Version 1.3. Washington, DC. Smithsonian: National Museum of Natural History. Retrieved from http://www.botany.si.edu/pacificislandbiodiversity/hawaiianflora/supplement.htm Last accessed 09/08/2017.

Wendel JF and Cronn RC. 2003. *Polyploidy and the Evolutionary History of Cotton*. Advances in Agronomy 78, pp. 139-186.

Wendel JF, Brubaker CL, and Percival AE. 1992. *Genetic Diversity in Gossypium hirsutum and the Origin of Upland Cotton*. American Journal of Botany 79, pp. 1291-1310. Retrieved from http://www.jstor.org/stable/2445058?seq=1#page_scan_tab_contents

Wendel JF, Brubaker CL, and Seelanan T. 2010. The Origin and Evolution of Gossypium. In: *Physiology of Cotton* (Springer Netherlands, eds. Steward JM, Oosterhuis D, Heitholt JJ, Mauney JR), pp. 1-18. Retrieved from http://link.springer.com/chapter/10.1007/978-90-481-3195-2_1

Wood DW, Setubal JC, Kaul R, Monks DE, Kitajima JP, Okura VK, Zhou Y, Chen L, Wood GE, Almeida NF, Jr., Woo L, Chen Y, Paulsen IT, Eisen JA, Karp PD, Bovee D, Sr., Chapman P, Clendenning J, Deatherage G, Gillet W, Grant C, Kutyavin T, Levy R, Li MJ, McClelland E, Palmieri A, Raymond C, Rouse G, Saenphimmachak C, Wu Z, Romero P, Gordon D, Zhang S, Yoo H, Tao Y, Biddle P, Jung M, Krespan W, Perry M, Gordon-Kamm B, Liao L, Kim S, Hendrick C, Zhao ZY, Dolan M, Chumley F, Tingey SV, Tomb JF, Gordon MP, Olson MV, and Nester EW. 2001. *The genome of the natural genetic engineer Agrobacterium tumefaciens C58*. Science 294, pp. 2317-2323. Retrieved from http://www.sciencemag.org/content/294/5550/2317.long

WSSA. 1998. *"Herbicide Resistance" and "Herbicide Tolerance" defined. (Technology Note).* Weed Technology 12, pp. 789. Retrieved from http://www.jstor.org/stable/3989101 Last accessed 04/09/2013.

Wu C, Huang J, Sun Y, and Yang D. 2002. *Mode of Action of 4-Hydroxyphenylpyruvate Dioxygenase Inhibition by Triketone-type Inhibitors*. Journal of Medicinal Chemistry 45, pp. 2222-2228. Retrieved from http://dx.doi.org/10.1021/jm010568y

Wunderlin RP, B. F. Hansen, A. R. Franck, and Essig FB. 2017. PlantAtlas.org: *Atlas of Florida Plants*. Retrieved from http://florida.plantatlas.usf.edu/ Last accessed 09/08/2017.

Xi Z, Wang Y, Bradley RK, Sugumaran M, Marx CJ, Rest JS, and Davis CC. 2013. *Massive mitochondrial gene transfer in a parasitic flowering plant clade*. Public Library of Genetics 9, pp. e1003265. Retrieved from http://www.plosgenetics.org/article/info%3Adoi%2F10.1371%2Fjournal.pgen.1003265 Last accessed 09/08/2017.

Xi Z, Bradley RK, Wurdack KJ, Wong KM, Sugumaran M, Bomblies K, Rest JS, and Davis CC. 2012. *Horizontal transfer of expressed genes in a parasitic flowering plant*. BMC Genomics 13, pp. 227-234. Retrieved from http://www.biomedcentral.com/1471-2164/13/227 Last accessed 09/08/2017.

York AC, Stewart AM, Vidrine PR, and Culpepper AS. 2004. *Control of volunteer glyphosate resistant cotton in glyphosate-resistant soybean*. Weed Technology 18, pp. 532-539.

Yoshida S, Maruyama S, Nozaki H, and Shirasu k. 2010. *Horizontal Gene Transfer by the Parasitic Plant Striga hermonthica*. Science. 328, p.1134. Retrieved from http://www.sciencemag.org/content/328/5982/1128.full

Zambryski P. 1988. *Basic Processes Underlying the Agrobacterium-mediated DNA Transfer to Plant Cells*. Annual Review of Genetics 22, pp. 1-30.

Zhang B, Pan X, Guo T, Wang Q, and Anderson TA. 2005. *Measuring gene flow in the cultivation of transgenic cotton (Gossypium hirsutum L.)*. Molecular Biotechnology 31, pp. 11-20.

Zhu B, Lou MM, Xie GL, Zhang GQ, Zhou XP, Li B, and Jin GL. 2011. *Horizontal gene transfer in silkworm, Bombyx mori*. BMC genomics 12, pp. 248-256. Retrieved from http://www.biomedcentral.com/1471-2164/12/248