Biotechnology Notification File No. 000161
Note to the File

Date: November 7, 2018

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To: Administrative Record, BNF No. 000161

Subject: Herbicide tolerant GHB811 cotton

Keywords:

Herbicide tolerant, GHB811, cotton, *Gossypium hirsutum*, BNF 000161, 5-enol pyruvylshikimate-3-phosphate synthase, 2mEPSPS, *Zea mays*, HPPD W336, *hppdPf W336-1Pa*, *Pseudomonas fluorescens*, glyphosate, p-hydroxyphenylpyruvate dioxygenase, HPPD, isoxaflutole, BCS-GH811-4, Bayer CropScience LP, BASF Agricultural Solutions Seed US LLC

Purpose

This document summarizes the Food and Drug Administration's (FDA's, our) evaluation of biotechnology notification file (BNF) No. 000161. Bayer CropScience LP (Bayer)[1] submitted a safety and nutritional assessment of genetically engineered (GE) herbicide tolerant cotton, transformation event GHB811 cotton (GHB11 cotton), which we received on April 26, 2017. We received additional information from Bayer on August 30, 2017. We evaluated the information in Bayer's submissions to ensure that regulatory and safety issues regarding human or animal food derived from GHB811 cotton have been resolved prior to commercial distribution.

In our evaluation, we considered all information provided by Bayer as well as publicly available information and information in the agency's files. Here we discuss the outcome of the consultation, but do not intend to restate the information provided in the final consultation in its entirety.

Intended Effects

The intended effect of the modifications in GHB811 cotton is herbicide tolerance to glyphosate and p-hydroxyphenylpyruvate dioxygenase (HPPD)-inhibiting herbicides. To confer tolerance to glyphosate, Bayer introduced the *2mepsps* gene from corn (*Zea mays*) that encodes the double mutant 5-enol pyruvylshikimate-3-phosphate synthase (2mEPSPS) protein. To confer

[1] During this consultation, event GHB811 cotton was divested by Bayer CropScience LP to BASF Agricultural Solutions Seed US LLC. This change was communicated by letter from BASF dated August 1, 2018.

tolerance to HPPD inhibitor herbicides, such as isoxaflutole, Bayer introduced the *hppdPf W336-1Pa* gene from *Pseudomonas fluorescens* that encodes the HPPD W336 protein.

Regulatory Considerations

The purposes of this evaluation are (1) to assess whether the developer has introduced into human and animal food a substance requiring premarket approval as a food additive and (2) to determine whether use of the new plant variety in human or animal food raises other regulatory issues under the Federal Food, Drug, and Cosmetic Act (FD&C Act).

The United States Environmental Protection Agency (EPA) regulates herbicides under the FD&C Act and the Federal Insecticide, Fungicide, and Rodenticide Act. Under EPA regulations, the herbicide residues in GHB811 cotton are considered pesticide residues. In its submission to FDA, Bayer stated that it has submitted a regulatory package to EPA for the use of glyphosate and HPPD-inhibitor herbicides on GHB811 cotton.

Genetic Modification and Characterization

Transformation Plasmid and Method

Bayer transformed hypocotyl segments from seedlings of cotton variety Coker 312 with transformation plasmid pTSIH09 using *Agrobacterium tumefaciens*, resulting in GHB811.

The transfer DNA (T-DNA) region in plasmid pTSIH09 contained two expression cassettes:

1. A *hppdPfW336-1Pa* expression cassette consisting of the *lox* sequence including the 34 base pair (bp) recognition sequence for the Cre recombinase of bacteriophage P1, the promoter sequence *pscsvmv* from the cassava vein mosaic virus, the *TPotpY-1Pa* coding sequence of an optimized transit peptide from *Z. mays* and *Helianthus annuus*, the *hppdpfW336-1Pa* coding sequence from *P. fluorescens*, and the 3′ untranslated region of the histone H3 gene from *Arabidopsis thaliana*.
2. A *2mepsps* expression cassette consisting of the promoter sequence *ph4a748* of the histone H4 gene from *A. thaliana*, the *intron1 h3At* sequence of gene II of the histone H3.III variant of *A. thaliana*, the *TPotpC* coding sequence of an optimized transit peptide from *Z. mays* and *H. annuus*, the *2mEPSPS* coding sequence from the double-mutant 5-enolpyruvylshikimate-3-phosphate synthase gene of *Z. mays*, the 3′ untranslated region of the histone H4 gene of *A. thaliana*, and the *lox* sequence including the 34 bp recognition sequence for the Cre recombinase of bacteriophage P1.

After transformation, the plants were treated with tembotrione (HPPD-inhibitor herbicide) to select for expression of the *hppdPfw336-1Pa* genes. The selected plants were then self-pollinated through seven generations and analyzed for resistance to glyphosate, presence of the inserted sequence, copy number, insertion integrity and absence of plasmid backbone sequences.

Characteristics, Inheritance, and Stability of the Introduced DNA

Bayer confirmed through Southern blot analysis that a single copy of the complete T-DNA of the pTSIH09 plasmid was inserted at a single locus of the GHB811 cotton genome. Bayer also further characterized the insertion by amplifying the flanking genomic DNA and T-DNA sequences using polymerase chain reaction (PCR) followed by direct sequencing; this was used

to confirm that the T-DNA was inserted into the cotton genome as well as to compare the identity of the inserted T-DNA with the sequence of the T-DNA region in pTSIH09. Southern blot analysis and sequencing also confirmed the absence of vector backbone sequences[2] in GHB811 cotton.

Bayer demonstrated the stability of the inserted DNA by describing consistent Southern blot hybridization results over five generations. Bayer also used PCR to determine the segregation ratios of the inserted DNA and Chi-square analysis to show that the insertion in GHB811 cotton is inherited according to Mendelian principles, consistent with a single chromosomal insertion.

Bayer used bioinformatic analyses to assess putative translation products of open reading frames (ORFs) generated by the inserted DNA and junctions comparing these ORFs to sequences of known toxic or allergenic proteins. Bayer reports that none of the putative ORFs would encode peptides of 30 amino acids or longer with similarity to any known allergens in the AllergenOnline public database or known toxins in the NCBI protein database.

Protein Characterization

Identity and Function of Introduced Protein

GHB811 cotton was genetically engineered to express the 2mEPSPS protein. The 2mEPSPS contains two amino acid substitutions compared to the native EPSPS from *Z. mays*. The resulting amino acid substitutions reduce the protein's binding affinity for glyphosate, which confers tolerance to the herbicide. Bayer notes that the 2mEPSPS protein has been previously reviewed in BNF 000109 and BNF 000122.

GHB811 cotton was also genetically engineered to express the HPPD W336 protein. The HPPD W336 protein was modified from the wild type *P. fluorescens* strain A32 HPPD protein by one amino acid substitution.[3] This substitution reduces binding affinity for HPPD-inhibiting herbicides, thereby conferring tolerance.

Protein Expression Level

Using enzyme-linked immunosorbent assays (ELISA),[4] Bayer measured expression of 2mEPSPS and HPPD W336 in GHB811 cotton tissues grown in 2015 at three field trial locations in the United States. These field trials were grown under typical commercial cotton production conditions, and each field trial had two plots, herbicide-treated (isoxaflutole and glyphosate) and herbicide-untreated. The mean 2mEPSPS expression levels in fuzzy cotton obtained from untreated and treated GHB811 cotton were 145.11 ± 37.86 µg/g dry weight and 150.88 ± 27.87 µg/g dry weight, respectively. The mean HPPD W336 expression levels in fuzzy seed obtained from untreated and herbicide-treated GHB811 cotton were 29.61 ± 14.96 µg/g dry weight and 27.01 ± 9.78 µg/g dry weight, respectively.

In discussing potential human and animal exposure, Bayer notes that HPPD W336 and the 2mEPSPS proteins were not detected in GHB811 cotton meal after toasting, crude oil, and refined, bleached, and deodorized oil samples.

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[2] The vector backbone is the region of pTSIH09 outside of the T-DNA region.

[3] Glycine at position 336 is changed to tryptophan.

[4] Bayer notes that the ELISAs used to measure protein expression in GHB811 cotton tissues were specific for 2mEPSPS and HPPD W336.

Potential for Toxicity and Allergenicity of the Introduced Proteins

Bayer notes that the safety of 2mEPSPS was discussed in BNF 000109 and 000122, and the safety of HPPD W336 was previously discussed in BNF 000122. Bayer presently reports heat stability, digestibility, and toxicity data from previous studies.[5] Bioinformatics analyses were updated for this submission.

Bayer describes bioinformatic analyses of 2mEPSPS and HPPD W336 proteins to assess whether they are similar to known toxins or allergens. Bayer found no structurally-relevant homology to known allergen or toxin sequences in the public database AllergenOnline (FARRP), NCBI non-redundant protein database, and Bayer's in-house toxin database.

Bayer assessed the heat stability of 2mEPSPS and HPPD W336. For 2mEPSPS, Bayer noted that there was no loss in mean specific activity up to 55°C, where the mean specific activity decreased, and minor visible degradation of the protein occurred. After treatment at 75°C and above, no specific activity for 2mEPSPS was detected. Bayer noted that HPPD W336 was structurally stable at temperatures up to 90°C; however, mean specific activity was halved after treatment at 45°C. After treatment at or above 60°C, no HPPD W336 activity was observed.

Bayer assessed the stability of each protein in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF). Both 2mEPSPS and HPPD W336 were degraded rapidly in both SGF and SIF.

Bayer reported results from acute oral toxicity studies in mice administered microbially produced 2mEPSPS and HPPD W336. Each study found no adverse effects up to and including the highest tested level.

Bayer concludes that the safety of the introduced 2mEPSPS and HPPD W336 proteins has been thoroughly evaluated and shown to pose no concerns.

Human and Animal Food Use

Cotton

Cotton (*Gossypium hirsutum* L.) is grown worldwide as a source of fiber for the textile industry. Cottonseed, which is a by-product of fiber production, is used in human food, animal food, and a range of industrial products. Food uses of cottonseed include cottonseed oil and cotton linters. Cottonseed oil is highly refined to remove naturally occurring toxicants, gossypol and cyclopropenoid fatty acids (CPFAs). Cottonseed oil is primarily consumed as a salad or cooking oil. Cotton linters are short fibers that remain on cotton seeds after ginning. They are removed from the seeds and processed into pure cellulose, which is used, for example, in casings for bologna, sausages, and frankfurters, and in other products such as ice cream or salad dressings.

Whole cottonseed, cottonseed meal, crude cottonseed oil, hulls, and cotton gin trash are used in animal food for cattle, sheep, goats, horses, poultry, swine, fish, or shrimp. Cottonseed meal

[5] Because the concentrations of 2mEPSPS and HPPD W336 in GHB811 cotton are low, Bayer conducted safety assessment studies using microbially-expressed 2mEPSPS and HPPD W336. Bayer demonstrated the equivalence of plant-expressed and microbially-expressed proteins using SDS-PAGE, western blot, glycosylation, mass spectrometry, and N-terminal sequence analyses.

is the product obtained from the flakes or cake after oil removal and is used as a protein supplement in animal food. Cottonseed hulls are used as a source of fiber in animal food.

Composition

Scope of Analysis

Bayer analyzed the composition of acid de-linted cottonseed from event GHB811 cotton, the parent variety (Coker 312), and seven non-GE commercial varieties that were grown and harvested under similar conditions. Three of seven reference varieties were grown at each field trial site.

Study Design - Compositional Analyses

Bayer conducted field trials during 2014 and 2015 at 15 field trials in cotton-producing areas throughout the United States. Event GHB811 cotton was grown both with isoxaflutole and glyphosate herbicide treatment (treated GHB811 cotton) and without (untreated GHB811 cotton). Four replicates of each entry were grown at each site using a randomized complete block design.

Composition data generated from eight sites were used for statistical analyses. Bayer notes that these eight sites selected of the original fifteen were selected based on their representativeness of cotton-growing regions in the U.S. and were selected prior to compositional analysis. Bayer notes that for components where greater than one-third of the values were below the limit of quantification (LOQ), Bayer excluded these components from statistical analysis.

Composition data for each component from treated GHB811 cotton, untreated GHB811 cotton, and control were combined by site and analyzed using analysis of variance approaches. Bayer compared levels of each component in both treated and untreated GHB811 cotton with the control using t-tests and a significance level of $p < 0.05$. Bayer calculated tolerance intervals for each component from the data obtained from reference varieties.[6] The ranges and tolerance intervals for the reference varieties were used by Bayer to assess whether differences in nutrient composition were biologically relevant.

Results of analyses

Bayer reported results for proximates (moisture, ash, carbohydrate by calculation, crude fat, and crude protein), fiber (acid detergent fiber, neutral detergent fiber, and total dietary fiber), minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium and zinc), anti-nutrients (free gossypol, total gossypol, dihydrosterculic acid, malvalic acid, and sterculic acid), 19 fatty acids, 18 amino acids, and alpha tocopherol. Bayer lists 15 components that were excluded from statistical analysis because over one-third of the values were below the LOQ.

Bayer reported finding statistically significant differences between the control and either treated GHB811 cotton or untreated GHB811 cotton in the levels of 10 components. However, Bayer notes that when statistical differences were observed, the mean values for these components in treated or untreated GHB811 cotton fell within the range of the reference varieties and within the tolerance intervals.

[6] The tolerance interval calculated by Bayer represents, with 95% confidence, 99% of the population of values from the reference varieties.

Summary of Compositional Analyses

Bayer states that the observed statistically significant differences between GHB811 cotton and the control are not biologically relevant because the means for all measured components in GHB811 cotton fell within the range of values for the reference varieties and within the tolerance intervals.

Conclusion

FDA evaluated Bayer's submission to determine whether GHB811 cotton raises any safety or regulatory issues with respect to its uses in human or animal food. Based on the information provided by the company and other information available to the agency, FDA did not identify any safety or regulatory issues under the FD&C Act that would require further evaluation at this time.

Bayer has concluded that its herbicide-tolerant cotton variety, GHB811 cotton, and the human and animal foods derived from it are not materially different in composition or any other relevant parameter from other cotton varieties now grown, marketed, and consumed. At this time, based on Bayer's data and information, the agency considers Bayer's consultation on GHB811 cotton to be complete.

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