Biotechnology Notification File No. 000172
CVM Note to the File

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To: Administrative Record, BNF No. 000172

Subject: Event GMB151 Soybean

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Purpose

This document summarizes the Food and Drug Administration (FDA) Center for Veterinary Medicine's (CVM, we) evaluation of biotechnology notification file (BNF) number 000172. BASF Corporation (BASF) submitted a safety and nutritional assessment for a genetically engineered (GE) soybean, transformation event GMB151 (hereafter referred to as GMB151 soybean) and additional information afterwards. CVM evaluated the information in BASF's submissions to ensure that regulatory and safety issues regarding animal food derived from GMB151 soybean have been resolved prior to commercial distribution. FDA's Center for Food Safety and Applied Nutrition summarizes its evaluation of GMB151 soybean in human food in a separate document.

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

Intended Effects

The intended effects of the modifications in GMB151 soybean are protection against plant-parasitic nematodes and tolerance to 4-hydroxyphenylpyruvate dioxygenase (HPPD)-inhibitor herbicides. To confer insect resistance, BASF introduced the *cry14Ab-1.b* gene from *Bacillus thuringiensis* that encodes for the Cry14Ab-1 protein. To confer tolerance to HPPD-inhibitor herbicides, BASF introduced a *hppdPf-4Pa* gene from *Pseudomonas fluorescens* that encodes a modified 4-hydroxyphenylpyruvate dioxygenase protein (HPPD-4). HPPD-4 served as a selectable marker.

Regulatory Considerations

The purpose of this evaluation is to determine whether use of the new plant variety in animal food raises safety or regulatory issues under the Federal Food, Drug and Cosmetic Act (FD&C Act).

The Environmental Protection Agency (EPA) defines a plant-incorporated protectant (PIP) as "a pesticidal substance that is intended to be produced and used in a living plant, or the produce thereof, and the genetic material necessary for the production of such a pesticidal substance," including "any inert ingredient contained in the plant, or produce thereof" (40 CFR 174.3). EPA regulates PIPs under the Federal Insecticide, Fungicide, and Rodenticide Act and the FD&C Act. Under EPA regulations, the Cry14Ab-1 protein is considered a pesticidal substance, and the HPPD-4 protein is considered an inert ingredient. Under EPA's regulations, Cry14Ab-1 and HPPD-4 proteins are subject to existing tolerance exemptions in 40 CFR 174.540[1] and 40 CFR 174.537, respectively.

The Environmental Protection Agency (EPA) also regulates herbicides under the FD&C Act and the Federal Insecticide, Fungicide, and Rodenticide Act. Under EPA regulations, the herbicide residues in GMB151 soybean are considered pesticidal residues.

Inheritance and Stability

BASF characterized the insertion event and genomic stability of the insert in the GMB151 soybean genome using bioinformatics techniques based on data obtained from whole genome sequencing (WGS), junction sequence analysis (JSA), and directed sequencing. BASF estimates that it collected sufficient data for WGS to cover the soybean genome at least 75-fold. Genomic DNA from the parental variety, Thorne, was also sequenced using WGS and was used as the comparator. BASF identified two "plasmid/flank" junction sequences and performed directed sequencing to determine the insertion site. BASF concludes that GMB151 soybean contains a single insert with one copy of the complete *cry14Ab-1.b* gene cassette and an incomplete copy of the *hppdPf-4Pa* gene cassette which lacks the 5' part of its promoter.

BASF performed WGS and JSA on four self-pollinated and one backcross generations to confirm the genomic stability. BASF detected identical junction sequences in each of the generations tested. In addition, BASF assessed inheritance in five generations (two F2 and three backcross generations) using event-specific polymerase chain reaction. The results of chi-square analysis of the segregation data from five generations show that the inheritance pattern of the insert is consistent with Mendelian principles of inheritance for a single locus. BASF concludes that the insert in GMB151 soybean was integrated at one locus and is stably transmitted across multiple generations.

Animal Food Use

Soybean (*Glycine max*) is grown around the world for a variety of human and animal food and industrial uses. Most soybean seeds are processed into oil and meal. Soybean

[1] BASF submitted a petition proposing a tolerance exemption for residues of Cry14Ab-1 in soybean to EPA. In June 2020, EPA granted a permanent exemption from the requirement of a tolerance for residues of the *Bacillus thuringiensis* Cry14Ab-1 in soybeans (40 CFR 174.540).

oil is commonly used as a human food ingredient. The preponderance of soybean meal is used in animal food, primarily in poultry, swine, and beef and dairy cattle diets. Soybean meal is processed in moist heat to inactivate trypsin inhibitors and lectins, which are anti-nutrients occurring in raw soybeans.

Composition

Scope of Analysis

BASF analyzed the nutrient composition of soybean forage and seed from GMB151 soybean, the conventional parental variety Thorne (control), and nine non-GE reference varieties (reference varieties) that were grown and harvested under similar conditions.

Study Design

BASF grew GMB151 soybean, the control, and reference varieties at eight sites in the United States in 2017. Soybean varieties were planted using a randomized complete block design with four replicate plots per site. Three of the nine reference varieties were grown at each field trial site. GMB151 soybean was grown both with HPPD-inhibitor herbicide treatment (treated GMB151 soybean) and without (untreated GMB151 soybean). BASF harvested forage and seed from each replicate within each site for compositional analysis. Forage and seed samples were collected at pod formation and seed maturity, respectively.

For the statistical analysis, BASF combined composition data for each component from treated GMB151 soybean, untreated GMB151 soybean, control, and reference varieties by site and analyzed these data using a mixed model analysis of variance approach (ANOVA). BASF excluded components from the analysis if over one third of the values were below the limit of quantification (LOQ). If fewer than one third of values were below the LOQ, BASF replaced values below the LOQ with a value equal to half of the LOQ.[2] BASF compared levels of each component in both treated and untreated GMB151 soybean with the control using t-tests and a significance level of $p < 0.05$. BASF calculated tolerance intervals for each component from the data obtained from the reference varieties.[3] The ranges and tolerance intervals for the reference varieties were used by BASF to assess whether differences in nutrient composition were biologically relevant.

Results of analyses

For forage, BASF reports values for moisture, proximates (crude protein, crude fat, carbohydrates by calculation, and ash), fiber (acid detergent fiber (ADF) and neutral detergent fiber (NDF)), calcium, and phosphorus. BASF found no statistically significant differences between the control and either treated or untreated GMB151 soybean in these components.

For the seed, BASF measured proximates, fiber (ADF, NDF, and total dietary fiber), 18 amino acids, 19 fatty acids, nine minerals, eight vitamins, four isoflavones (daidzein,

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[2] For seed composition, BASF lists 17 components that were excluded from statistical analysis because over one-third of the values were below the LOQ.

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

genistein, glycitein, and total), and five anti-nutrients. BASF reports statistically significant differences between the control and either treated or untreated GMB151 soybean in the levels of 22 components.[4] The mean values for 19 of these 22 components in treated or untreated GMB151 soybean fell within the mean ranges of the reference varieties and within the tolerance intervals. The mean values of C16:0 palmitic acid, C17:1 heptadecenoic acid, and C22:0 behenic acid fell outside the mean ranges of the reference varieties, but within the ranges of the tolerance intervals. Additionally, BASF states that the mean values of these three fatty acids were within the ranges present in the International Life Sciences Institute Crop Composition Database (ILSI-CCDB), version 6.[5] BASF concludes that the differences are not biologically relevant.

Summary of Compositional Analyses

BASF states based on the results from the compositional analyses that forage and seed obtained from GMB151 soybean are comparable to those of the control and reference varieties. BASF concludes that forage and seed obtained from GMB151 soybean are comparable in nutrition to conventional soybean varieties.

Conclusion

CVM evaluated BASF's submissions to determine whether GMB151 soybean raises any safety or regulatory issues with respect to its use in animal food. Based on the information provided by BASF and other information available to the agency, CVM did not identify any safety or regulatory issues under the FD&C Act that would require further evaluation at this time.

BASF concludes that GMB151 soybean and the animal foods derived from it are as safe as and are not materially different in composition or any other relevant parameter from other soybean varieties now grown, marketed, and consumed. At this time, based on BASF's data and information, CVM considers BASF's consultation on GMB151 soybean for use in animal food to be complete.

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[4] The 22 components are moisture, carbohydrates, crude protein, NDF, calcium, copper, zinc, vitamin B1 (thiamine), vitamin B5 (pantothenic acid), vitamin B9 (folic acid), phytic acid, total daidzein, total genistein, total glycitein, total isoflavones, and the fatty acids: C16:0 palmitic acid, C17:1 heptadecenoic acid, C18:1 oleic acid, C18:2 linoleic acid, C20:1 eicosenoic acid, C22:0 behenic acid, and C24:0 lignoceric acid.

[5] The ILSI Crop Composition Database has become the Agriculture and Food Systems Institute Crop Composition Database and is available at www.cropcomposition.org.