FDA U.S. FOOD & DRUG ADMINISTRATION
CENTER FOR VETERINARY MEDICINE

Biotechnology Notification File No. 000202
CVM Note to the File

Date: May 30, 2025

From: Ramavati Pal, Ph.D.

To: Administrative Record, BNF No. 000202

Subject: Event MON 89151 Cotton

Keywords: Cotton, *Gossypium hirsutum* (L.), *cry1Da_7* gene, Cry1Da_7 protein, *cry1B.3* gene, Cry1B.3 protein, *Bacillus thuringiensis, vip3Cb1.1* gene, Vip3Cb1.1 protein, *Paenibacillus* bacterium, Lepidopteran insect resistant, OECD Identifier MON-89151-3, Bayer CropScience LP.

Purpose

This document summarizes the Food and Drug Administration (FDA) Center for Veterinary Medicine's (CVM, we) evaluation of biotechnology notification file (BNF) number 000202. Bayer CropScience LP (Bayer) submitted a safety and nutritional assessment for a genetically engineered (GE) cotton, transformation event MON-89151-3 (hereafter referred to as MON 89151 cotton). CVM evaluated the information in Bayer's submission to ensure that regulatory and safety issues regarding animal food derived from MON 89151 cotton have been resolved prior to commercial distribution. FDA's Human Foods Program (HFP) summarizes its evaluation of MON 89151 cotton in human food in a separate document.

In CVM's evaluation, we considered all of the 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 for animal food use, 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 MON 89151 cotton is to provide resistance to certain lepidopteran pests including cotton bollworm, tobacco budworm, old world bollworm, Australian budworm, fall armyworm and soybean looper. To confer the insect resistance trait, Bayer introduced the *cry1Da_7* and *cry1B.3* genes from *Bacillus thuringiensis* that encode for the Cry1Da_7 and Cry1B.3[1] proteins, respectively and *vip3Cb1.1* gene from *Paenibacillus* bacterium that encodes for Vip3Cb1.1 protein. In addition, Bayer also introduced the selection marker *aad*A cassette and the *cre* cassette

[1] Cry1B.3 is a chimeric protein comprised of domains I and II from Cry1Be, domain III from Cry1Ka2 and a protoxin domain from Cry1Be.

flanked by *loxP* sites.[2] Bayer states that in *cre/lox*-mediated auto-excision system, the selectable markers are removed in a single step during the development of R1 seeds.[3]

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 (FIFRA) and the FD&C Act. Under EPA regulations, the Cry1Da_7, Cry1B.3 and Vip3Cb1.1 proteins and the genetic material used to express them in MON 89151 cotton are considered to be pesticidal substances. Therefore, the safety assessment of these products falls under the regulatory purview of EPA.

Stability and Inheritance

Bayer characterized the insertion event and genomic stability of the insert in the MON 89151 cotton genome using whole genome sequencing (WGS) and subsequent read mapping method. To confirm genomic stability, Bayer performed WGS on multiple breeding generations (R2, R3, R4, R5, and R6) of MON 89151 cotton. Bayer detected two identical junction sequences in each of the generations tested, indicating a single site of insertion, and no junction sequences were observed in the non-GE control line.[4]

In addition, Bayer assessed inheritance of the inserted transfer DNA in MON 89151 cotton in R2F2, R2F3 and R2F4 generations using quantitative PCR. The results of Chi-square analysis of the segregation data from three generations show that the segregation pattern of the insert is consistent with Mendelian principles of inheritance for a single locus. Bayer concludes that the insert in MON 89151 cotton was integrated at one locus and is stably transmitted across multiple generations.

Animal Food Use

Following cleaning and ginning, whole cottonseed (after removal of the cotton fibers) is processed into four major human and animal food products: oil, linters, meal, and hulls. The first two ingredients are almost exclusively used in human food. Whole cottonseed, acid delinted cottonseed, cottonseed meal, hulls, and cotton gin trash are used as ingredients in food for ruminant animals.[5] The amount of cottonseed meal that can be

---

[2] The *aadA* gene that encodes for an aminoglycoside-modifying enzyme, 3"(9)-O- nucleotidyltransferase from the transposon Tn7 which confers spectinomycin and streptomycin resistance.

[3] Bayer states a line without selectable marker and *cre* cassette was used as MON 89151 cotton which contained T-DNA I (with the *cry1Da_7*, *cry1B.3* and *vip3Cb1.1* expression cassettes and without any plasmid vector backbone).

[4] Bayer used non-GE conventional cotton variety DP393 with similar genetic background to the test variety as a control.

[5] NCPA. 2002. Cottonseed and its products. National Cottonseed Products Association, Cordova, Tennessee. http://www.cottonseed.com/publications/cottonseedanditsproducts.asp [Accessed

used in monogastric animal diets is normally limited by the presence of gossypol. Cottonseed meal which contains not more than 0.04% (400 parts per million (ppm)) free gossypol, can be used as a source of protein in food for animals. For example, cottonseed meal from glandless varieties of cotton have been used in animal food for monogastric species, such as swine and poultry, and aquaculture.[6] Bayer references the Organisation for Economic Co-operation and Development (OECD) consensus document[7] on biology of cotton and the use of cotton as a crop plant.[8]

Composition

Scope of Analysis

Bayer analyzed the nutrient composition of MON 89151 cotton and conventional control cotton. All were grown and harvested under similar agronomic field conditions. Compositional analyses on cottonseed samples were reported for components listed in the OECD cotton composition consensus document.[9]

Study Design

Bayer conducted field trials in 2023 at five sites in the United States. A randomized complete block design with five replicate plots at each field site was used. Each block included MON 89151 cotton and non-GE conventional control cotton (DP393) and were grown under normal agronomic field conditions for their respective regions. Bayer harvested cottonseed samples from each replicate within each site for composition analysis. Cottonseed samples were harvested at physiological maturity and shipped at ambient temperature from the field sites to Bayer (where they were ginned). The samples were subsequently acid-delinted and a subsample for compositional analysis was obtained from each tissue sample collected. These subsamples were ground and stored in a freezer at -20°C. The samples were shipped on dry ice to analytical lab for nutrient compositional analysis.

For statistical analysis, Bayer combined composition data for each component from MON 89151 cotton and the control cotton across locations using a linear mixed model with site and replicate as random factors. Mixed model analyses were used to test at the level of $P<0.05$ for differences between MON 89151 cotton and the conventional control cotton. Bayer states that in order to complete a statistical analysis for a compositional constituent in compositional assessment, at least 50% of all the values for an analyte in cottonseed had to be greater than the assay limit of quantitation (LOQ). Analytes with more than 50% of observations below the assay LOQ were excluded from summaries

---

October 17, 2011].

[6] Low gossypol cottonseed meal, which contains not more than 0.04% free gossypol, that was obtained by mechanical or solvent extraction are defined (24.50 and 24.51, respectively) in the Official Publication of the Association of American Feed Control Officials as ingredients for use in food for animals.

[7] OGTR. 2024. The biology of *Gossypium hirsutum* L. and *Gossypium barbadense* L. (cotton). Version 3.1. Australian Government, Department of Health and Aged Care, Office of the Gene Technology Regulator, Canberra, Australia.

[8] OECD. 2008. Consensus document on the biology of cotton (Gossypium spp.). ENV/JM/MONO (2008)33. Organisation for Economic Co-operation and Development, Paris, France

[9] OECD. 2009. Consensus document on the compositional considerations for new varieties of cotton (*Gossypium hirsutum* and *Gossypium barbadense*): Key food and feed nutrients and anti-nutrients.

and statistical analysis. When a statistically significant difference was identified, it was further evaluated to determine if the difference indicated a biologically relevant compositional change or supported a conclusion of compositional equivalence based on 1) difference in means between MON 89151 cotton and conventional control cotton; 2) difference in the context of natural variation within the conventional control cotton across multiple sites and 3) difference in the context of natural variation due to multiple sources such as environmental and non-GE control DP393 influences. This assessment determined whether the component mean value of MON 89151 cotton was within the non-GE varieties defined by the literature values[10] or the Agriculture and Food Systems Institute Crop Composition Database (AFSI CCDB) values.[11] Results were all expressed on a dry matter basis prior to statistical analyses except for fatty acids, which were expressed on a percent of total fatty acids basis.

Results of Analyses

Bayer chemically analyzed proximates (protein, total fat, carbohydrates by calculation and ash), fiber (Acid detergent Fiber (ADF), Neutral Detergent Fiber (NDF) and Total Detergent Fiber (TDF)), moisture, 18 amino acids, seven fatty acids, two minerals (calcium, and phosphorus), vitamin (vitamin E), five anti-nutrients (total gossypol, free gossypol, dihydrosterculic acid, malvalic acid and sterculic acid). Bayer noted that none of the 41 measured components had more than 50% of the observations below the assay LOQ. Except moisture, all 40 components were statistically analyzed. Bayer reports statistically significant differences between the control cotton and MON 89151 cotton in the levels of 10 components (protein, tryptophan, myristic acid, palmitic acid, palmitoleic acid, oleic acid, carbohydrates by calculation, malvalic acid, sterculic acid and dihydrosterculic acid).

For these significantly different components in cottonseed, the mean difference values between MON 89151 cotton and the control cotton were within the range of values of non-GE varieties observed in the literature and/or the AFSI-CCDB with a history of safe use. Bayer concludes that the differences in these components between MON 89151 cotton and the control cotton are not biologically meaningful from an animal food safety perspective.

---

[10] Literature range references:
Hamilton, K.A *et al.*2004. Bollgard II cotton: Compositional analysis and feeding studies of cottonseed from insect-protected cotton (*Gossypium hirsutum* L.) producing the Cry1Ac and Cry2Ab2 proteins. Journal of Agricultural and Food Chemistry 52:6969-6976.
Harrison, J.M. *et al.* 2013. Principal variance component analysis of crop composition data: A case study on herbicide-tolerant cotton. Journal of Agricultural and Food Chemistry 61:6412-6422.

[11] AFSI CCDB. 2024. Crop Composition Database. Agriculture & Food Systems Institute (Accessed May 23, 2024).

Summary of Compositional Analyses

Bayer states based on the results from the compositional analyses, that cottonseed samples obtained from MON 89151 cotton are not biologically different from those of the control varieties. Bayer concludes that these results support the conclusion that cottonseed obtained from MON 89151 cotton are compositionally comparable to the control in the levels of key nutrients, anti-nutrients including gossypol and cyclopropenoid fatty acid.

Conclusion

CVM evaluated Bayer's submission to determine whether MON 89151 cotton raises any safety or regulatory issues with respect to its uses in animal food. Based on the information provided by Bayer 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.

Bayer concludes that MON 89151 cotton 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 cotton varieties now grown, marketed, and consumed. At this time, based on Bayer's data and information, CVM considers Bayer's consultation on MON 89151 cotton for use in animal food to be complete.

RAMAVATI R. PAL -S

Digitally signed by RAMAVATI R. PAL -S
Date: 2025.06.06 15:10:33 -04'00'

Ramavati Pal, Ph.D.
Staff Fellow Biologist