

Biotechnology Notification File No. 000195 CVM Note to the File

Date: March 17, 2025

From: Lei Dai, BVSc, Ph.D.

To: Administrative Record, BNF No. 000195

Subject: Event MON 94804 Corn

Keywords: Corn, Maize, *Zea mays* L., GA20ox_SUP miRNA, RNA interference, Short stature, OECD Unique Identifier MON-94804-4, 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 000195. Bayer CropScience LP. (Bayer) submitted a safety and nutritional assessment for a genetically engineered (GE) corn, transformation event MON 94804 (hereafter referred to as MON 94804 corn). CVM evaluated the information in Bayer's submission to ensure that regulatory and safety issues regarding animal food derived from MON 94804 corn have been resolved prior to commercial distribution. FDA's Human Foods Program summarizes its evaluation of uses of MON 94804 corn 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 modification in MON 94804 corn is to reduce internode length and consequently reduce overall plant height in the GE corn variety. To confer the short stature trait, Bayer introduced an expression cassette encoding the GA20ox_SUP micro ribonucleic acid (GA20ox_SUP miRNA) targeting the messenger RNA (mRNA) transcribed from the *ZmGA20ox3* and *ZmGA20ox5* genes¹ in corn to reduce production of plant hormone gibberellin transcripts, through an RNA interference (RNAi) process.

¹ Bayer states that *ZmGA20ox3* and *ZmGA20ox5* genes are two of nine *ZmGA20ox* genes in corn genome that encode the gibberellin 20 oxidase which is a key enzyme in synthesizing bioactive gibberellins in the gibberellin biosynthesis pathway. Bayer further states that expression levels of *ZmGA20ox3* and *ZmGA20ox5* genes are relatively higher in vegetative tissues and lower in reproductive tissues, compared to other *ZmGA20ox* genes.

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 U.S. Environmental Protection Agency (EPA) evaluates and authorizes the use of plant incorporated protectants (PIPs) under the FD&C Act and the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). A PIP is defined in 40 CFR 174.3 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.” The term pesticide, as defined in FIFRA section 2(u) includes “any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest; any substance or mixture of substances intended for use as a plant regulator, defoliant, or desiccant; and any nitrogen stabilizer.”

Genetic Modification and Characterization

Introduced DNA and Transformation Method

Bayer developed MON 94804 corn from mature embryo explants of corn inbred line HCL301 through *Agrobacterium tumefaciens* mediated transformation with plasmid PV-ZMAP527892. The transfer-DNA (T-DNA) region within plasmid PV-ZMAP527892 contains sequence coding for GA20ox_SUP miRNA, which is preceded by promoter and leader sequences, P²-RTBV-1, from Rice tungro bacilliform virus, and intron and flanking exon sequences of the *hsp70* gene from corn. Additionally, GA20ox_SUP miRNA coding sequence is followed by a 3' untranslated region sequence, T⁴-GST43, from corn. The T-DNA region also contains a selectable marker, 5-enolpyruvylshikimate-3-phosphate synthase (*cp4 epsps*) gene from *A. tumefaciens* strain CP4, coding for CP4 EPSPS protein that confers tolerance to the herbicide glyphosate. The *cp4 epsps* expression cassette is flanked by *loxP* excision targeting sequences. After transformation, explants were grown on media containing glyphosate to select for successful transformants. Subsequently, Cre/*lox*-mediated recombination was used to remove the *cp4 epsps* expression cassette through crossing with a corn line expressing Cre recombinase. Bayer states that *cre* gene and associated genetic elements coding for Cre recombinase were then segregated away from the GA20ox_SUP expression cassette by conventional breeding to produce MON 94804 corn.

Bayer characterized the insertion event of the insert in the MON 94804 corn genome using bioinformatics techniques based on data obtained from whole genome sequencing (WGS), junction sequence analysis, and directed sequencing. Based on these analyses, Bayer confirmed the absence of sequences of plasmid backbone, *cp4 epsps* expression cassette, and *cre* gene in the MON 94804 corn genome. To confirm genomic stability, Bayer performed WGS on multiple breeding generations of MON 94804 corn. 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 control lines.

In addition, Bayer assessed inheritance of the inserted transfer DNA in MON 94804 corn in three generations of hemizygous plants using Real-Time polymerase chain

reaction. 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 94804 corn was integrated at one locus and is stably transmitted across multiple generations.

Bayer performed bioinformatics analyses using the nucleotide sequences obtained for the inserted DNA and their corresponding flanking genomic junction sequences to determine whether insertion of the introduced DNA created any potential open reading frames that could code for putative polypeptides. Bayer reports that none of the putative polypeptides had significant identity with proteins in its toxin database, TOX_2024², and that a search in its protein database, PRT_2024, did not identify any unintended polypeptides generated at the insertion site. Based on the results of bioinformatics analyses, Bayer concludes that the T-DNA insertion does not lead to the production of putative polypeptides that would raise animal food safety concerns.

Animal Food Use

Bayer states that MON 94804 corn is suitable for any animal food uses of conventional corn. Bayer references the Organisation for Economic Co-operation and Development (OECD) consensus document on compositional considerations of maize³ and states that corn (*Zea mays* L.) is a commodity crop grown worldwide for various uses. Corn and its processed fractions have also been used in foods for animals. Bayer states that the production and different methods of processing as well as uses are described in greater detail in the OECD consensus document.

Composition

Scope of Analysis

Bayer analyzed the nutrient composition of MON 94804 corn and a non-GE corn with a similar genetic background (control) that were grown and harvested under similar conditions. Compositional analyses on grain and forage samples were reported for components listed in the OECD maize composition consensus document.

Study Design

Bayer conducted field trials in 2020 at five sites in the United States. A randomized complete block design with four replicate plots at each field site was used. The MON 94804 corn and control were grown under normal agronomic field conditions for their respective regions. Bayer harvested grain and forage from each replicate within each site for composition analysis. Grain was harvested at physiological maturity and shipped at ambient temperature from the field sites. Forage samples were harvested at R5 growth stage and were shipped on dry ice from the field sites to Bayer. A subsample for compositional analysis was obtained from grain and forage samples from each replicate at each site and stored at -20°C prior to nutrient analyses.

² According to Bayer, TOX_2024 is a subset of sequences derived from the Swiss-Prot database that was selected using a keyword search and filtered to exclude non-toxin proteins.

³ Organisation for Economic Co-operation and Development. 2002. Consensus document on compositional considerations for new varieties of maize (*Zea mays*): Key food and feed nutrients, antinutrients, and secondary plant metabolites. OECD ENV/JM/MONO 25. OECD, Paris, France.

For statistical analysis, Bayer combined composition data for each component from MON 94804 corn and the control across locations using a linear mixed model with site and replicate as random factors. T-test analyses were used to test at the level of $P \leq 0.05$ for differences between MON 94804 corn and control. Differences between MON 94804 corn and control were evaluated based on natural variability defined by values for conventional corn varieties in the Agriculture & Food Systems Institute (AFSI) Crop Composition Database (CCDB) or in the scientific literature. 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. Moisture of forage and grain were not statistically analyzed.

Results of Analyses

For forage, Bayer reports values for proximates (crude protein, crude fat, carbohydrates by calculation, and ash), fiber (acid detergent fiber (ADF), acid detergent lignin (ADL), and neutral detergent fiber (NDF)), calcium, and phosphorus. Bayer reports statistically significant differences between the MON 94804 corn and control in the levels of six components (total protein, carbohydrates by calculation, ADF, ADL, NDF, and ash).⁴ For these components, the mean difference between MON 94804 corn and the control was less than the range of values for the control and within the mean ranges of the non-GE varieties observed in the literature and/or the AFSI CCDB. Bayer concludes that the differences in these components between MON 94804 corn and the control are not biologically meaningful from an animal food safety perspective.

For grain, Bayer chemically analyzed proximates, fiber (ADF, ADL, NDF, and total dietary fiber (TDF)), 18 amino acids, 22 fatty acids, nine minerals, seven vitamins, two anti-nutrients and three secondary metabolites. Bayer noted that 13 of the fatty acids, sodium, and furfural were not statistically analyzed because more than 50% of the observations fell below the lower limits of quantitation (LOQ). Bayer reports statistically significant differences between the MON 94804 corn and control in the levels of four components (stearic acid, TDF, calcium, and ferulic acid). For these components, the mean difference between MON 94804 corn and the control was less than the range of values for the control and within the mean ranges of the non-GE varieties observed in the literature and/or the AFSI CCDB. Bayer concludes that the differences in these components between MON 94804 corn and the control are not biologically meaningful from an animal food safety perspective.

Summary of Compositional Analyses

Bayer states based on the results from the compositional analyses that forage and grain obtained from MON 94804 corn are not biologically different from those of the control and publicly available data representative of natural variation. Bayer emphasizes that these results support the conclusion that forage and grain obtained from MON 94804 corn are compositionally comparable to the control in the levels of key nutrients, anti-nutrients, and secondary metabolites.

⁴ Bayer states that crude protein and ash were statistically higher, while carbohydrates by calculation, ADF, ADL, and NDF were statistically lower in forage of MON 94804 corn compared to conventional control.

Conclusion

CVM evaluated Bayer's submissions to determine whether MON 94804 corn raises any safety or regulatory issues with respect to its use 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 94804 corn 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 corn varieties now grown, marketed, and consumed. At this time, based on Bayer's data and information, CVM considers Bayer's consultation on MON 94804 corn for use in animal food to be complete.

Lei Dai -S Digitally signed by Lei Dai -S
Date: 2025.03.18 11:56:57
-04'00'

Lei Dai, BVSc, Ph.D.
Biologist