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

Biotechnology Notification File No. 000199
CVM Note to the File

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From: Ramavati Pal, Ph.D.

To: Administrative Record, BNF No. 000199

Subject: Event MON 94637

Keywords: Soybean, *Glycine max* (L.) Merr., *cry1A.2* gene, Cry1A.2 protein, *cry1B.2* gene, Cry1B.2 protein, *Bacillus thuringiensis*, Insect resistant, OECD Identifier MON-94637-8, Bayer.

Purpose

This document summarizes the Food and Drug Administration (FDA) Center for Veterinary Medicine's (CVM, we) evaluation of biotechnology notification file (BNF) number 000199. Bayer submitted a safety and nutritional assessment for a genetically engineered (GE) soybean, transformation event MON-94637-8 (hereafter referred to as MON 94637 soybean), and additional information afterwards. CVM evaluated the information in Bayer's submissions to ensure that regulatory and safety issues regarding animal food derived from MON 94637 soybean have been resolved prior to commercial distribution. FDA's Human Foods Program (HFP) summarizes its evaluation of MON 94637 soybean 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 94637 soybean is to provide resistance to certain lepidopteran soybean pests including soybean looper and velvetbean caterpillar. To confer the insect resistance trait, Bayer introduced the *cry1A.2* and

cry1B.2 genes from *Bacillus thuringiensis* that encode for the Cry1A.2[1] and Cry1B.2[2] proteins, respectively.[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 Cry1A.2 and Cry1B.2 proteins and the genetic material used to express it in MON 94637 soybean are considered pesticidal substances, and the sucrose phosphorylase and 3"(9)-O-nucleotidyltransferase proteins and the genetic material used to express them are considered to be inert ingredients. 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 94637 soybean genome using whole genome sequencing (WGS) and subsequent read mapping method. To confirm genomic stability, Bayer performed WGS on five multiple breeding generations (R3, R4, R5, R6, and R7) of MON 94637 soybean. 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.[4]

In addition, Bayer assessed inheritance of the inserted transfer DNA in MON 94637 soybean in F2, F3 and F4 generations using Qualitative End Point TaqMan® PCR Lox-Mt1 assay. 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 94637 soybean was integrated at one locus and is stably transmitted across multiple generations.

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[1] Cry1A.2 is a chimeric protein comprised of domains I from Cry1Ah, domain II and the C-terminal domain from Cry1Ac, and domain III from Cry1Ca.

[2] Cry1B.2 is a chimeric protein comprised of domains I and II from Cry1Be, domain III from Cry1Ka2, and the C-terminal domain from Cry1Ab.

[3] In addition, Bayer also introduced the *splA* gene from *Agrobacterium tumefaciens* strain C58 that encodes the sucrose phosphorylase protein which catalyzes the conversion of sucrose to fructose and glucose–1–phosphate and *aadA* gene that encodes for an aminoglycoside-modifying enzyme, 3"(9)-O-nucleotidyltransferase from the transposon Tn7 which confers spectinomycin and streptomycin resistance. Bayer states that both sucrose phosphorylase and 3"(9)-O-nucleotidyltransferase proteins serve as selectable markers. Bayer states the plants with selectable marker genes *splA* and *aadA* or the backbone sequences from the transformation vector will not be used. Only plants homozygous for T-DNA I (containing the Cry1A.2 and Cry1B.2 expression cassettes) were subjected to further development.

[4] The conventional crop variety, which is a genetic background to the test substances.

Animal Food Use

Soybean 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 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. Bayer references the Organisation for Economic Co-operation and Development (OECD) consensus document[5] on biology of soybean and the use of soybean as a crop plant.

Composition

Scope of Analysis

Bayer analyzed the nutrient composition of MON 94637 soybean and conventional control soybean. All were grown and harvested under similar agronomic field conditions. Compositional analyses on grain and forage samples were reported for components listed in the OECD soybean composition consensus document.[6]

Study Design

Bayer conducted field trials in 2021 at five sites in the United States. A randomized complete block design with four replicate plots at each field site was used. Each block included MON 94637 soybean and conventional control and 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. Forage samples were harvested at R6 growth stage. Grain samples from the field sites were shipped at ambient temperature while forage samples were placed on dry ice. The samples were ground and stored in a freezer at -20°C. The samples were shipped on dry ice from each replicate at each site to analytical lab for nutrient compositional analyses.

For statistical analysis, Bayer combined composition data for each component from MON 94637 soybean and the control 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 94637 soybean and the conventional control. 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 grain or forage 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 and statistical analysis. When a statistically significant difference was identified, it was further evaluated to determine if the difference indicated a biologically

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[5] OECD. 2000. Consensus document on the biology of Glycine max (L.) Merr. (Soybean). ENV/JM/MONO (2000)9. Series on Harmonization of Regulatory Oversight in Biotechnology No.15.Paris, France, Organisation for Economic Co-operation and Development.

[6] OECD. 2012. Revised consensus document on compositional considerations for new varieties of soybean [Glycine max (L.) Merr.]: Key food and feed nutrients, anti-nutrients, toxicants and allergens. ENV/JM/MONO (2012)24. Series on the Safety of Novel Foods and Feeds No. 25. Paris, France, Organisation for Economic Co-operation and Development

relevant compositional change or supported a conclusion of compositional equivalence based on 1) difference in means between MON 94637 and conventional control; 2) difference in the context of natural variation within the conventional control across multiple sites and 3) difference in the context of natural variation due to multiple sources such as environmental and germplasm influences. This assessment determined whether the component mean value of MON 94637 was within the non-GE varieties defined by the literature values[7] or the Agriculture and Food Systems Institute Crop Composition Database (AFSI CCDB) values[8]. 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

For forage, Bayer reports values for proximates (crude protein, crude fat, carbohydrates by calculation, and ash), fiber (acid detergent fiber (ADF) and neutral detergent fiber (NDF)). Bayer found statistically significant difference ($p<0.05$) between the conventional control and MON 94637 soybean in two components (protein and carbohydrates by calculation).

For grain, Bayer chemically analyzed proximates (carbohydrates by calculation, ash), fiber (ADF and NDF), moisture, 18 amino acids, 22 fatty acids, two minerals (calcium, and phosphorus), two vitamins (vitamin E and vitamin K1), five anti-nutrients (phytic acid, raffinose, soybean lection, stachyose and trypsin inhibitor) and three isoflavones (daidzein, genistein and glycitein). Bayer noted that 11 of the fatty acids[9] were not statistically analyzed because more than 50% of the observations fell below the LOQ[10]. Bayer reports statistically significant differences between the control and MON 94637 grain in the levels of three components (palmitoleic acid, heptadecanoic acid and behenic acid).

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[7] Literature range references:
Lundry, D. R., W. P. Ridley, J. J. Meyer, S. G. Riordan, M. A. Nemeth, W. A. Trujillo, M. L. Breeze and R. Sorbet. 2008. Composition of grain, forage, and processed fractions from second-generation glyphosate-tolerant soybean, MON 89788, is equivalent to that of conventional soybean (Glycine max L.). Journal of Agricultural and Food Chemistry 56(12):4611-4622;
Berman, K. H., G. G. Harrigan, S. G. Riordan, M. A. Nemeth, C. Hanson, M. Smith, R. Sorbet, E. Zhu and W. P. Ridley. 2009. Compositions of seed, forage, and processed fractions from insect-protected soybean MON 87701 are equivalent to those of conventional soybean. Journal of Agricultural and Food Chemistry 57:11360-11369;
Bellaloui, N., J. R. Smith, A. M. Gillen and J. D. Ray. 2011. Effects of maturity, genotypic background, and temperature on seed mineral composition in near-isogenic soybean lines in the early soybean production system. Crop Science 51(3):1161-1171;
Thompson, M. M., A. Niemuth, J. Sabbatini, D. Levin, M. L. Breeze, X. Li, T. Perez, M. Taylor and G. G. Harrigan. 2016. Analysis of vitamin K1 in soybean seed: Assessing levels in a lineage representing over 35 years of breeding. Journal of the American Oil Chemists' Society Online:1-8 ;
Codex Alimentarius. 2021. Standard for named vegetable oils: CXS 210-1999. Codex Alimentarius Commission, Joint FAO/WHO Food Standards Programme, Food and Agriculture Organization of the United Nations, Rome, Italy.

[8] AFSI CCDB, 2022 (Accessed February 22, 2022).

[9] Caprylic acid, capric acid, lauric acid, myristoleic acid, pentadecanoic acid, pentadecenoic acid, heptadecenoic acid, gamma linolenic acid, eicosadienoic acid, eicosatrienoic acid and arachidonic acid.

[10] Lower Limit of Quantitation

For these significantly different components in forage and grain, the mean difference between MON 94637 soybean and the control was less than the range of values for the control and within the mean ranges of the non-GE varieties with a history of safe use. Bayer concludes that the differences in these components between MON 94637 soybean 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 94637 soybean are not biologically different from those of the control varieties. Bayer concludes that these results support the conclusion that forage and grain obtained from MON 94637 soybean are compositionally comparable to the control in the levels of key nutrients, anti-nutrients, and isoflavones.

Conclusion

CVM evaluated Bayer's submissions to determine whether MON 94637 soybean 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 94637 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 Bayer's data and information, CVM considers Bayer's consultation on MON 94637 soybean for use in animal food to be complete.

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