



## Biotechnology Notification File No. 000203 CVM Note to the File

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**To:** Administrative Record, BNF No. 000203

**Subject:** Event MON 94115 Soybean

**Keywords:** Soybean, *Glycine max* (L.) Merr., *H\_N90 PPO*, *Enterobacter cloacae*, protoporphyrinogen IX oxidase, Tolerance to PPO-inhibiting herbicides, OECD identifier MON-94115-8, 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 000203. Bayer CropScience LP (Bayer) submitted a safety and nutritional assessment for a genetically engineered (GE) soybean, transformation event MON 94115 (hereafter referred to as MON 94115 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 94115 soybean have been resolved prior to commercial distribution. FDA's Human Foods Program summarizes its evaluation of uses of MON 94115 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 modification in MON 94115 soybean is to confer tolerance to PPO-inhibiting herbicides. Bayer states that the parental soybean variety was transformed with the *protoporphyrinogen IX oxidase* (*H\_N90 PPO*) gene from *Enterobacter cloacae*, which encodes the PPO protein that confers tolerance to PPO-inhibiting herbicides.

## Regulatory Considerations

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

## Genetic Modification and Characterization

### Introduced DNA and Transformation Method

Bayer transformed meristem explants obtained from a non-genetically engineered soybean variety A3555 with vector PV-GMHT533023 using disabled *Agrobacterium tumefaciens* mediated transformation. Bayer states that the transfer-DNA (T-DNA) region within the plasmid contained four expression cassettes between the left (LB) and right (RB) border sequences. These include:

- *aadA* selectable marker cassette.
- *cre* recombinase cassette.
- site directed integration (SDI) [*Cpf1* (*Cas12a*) and gRNA] cassettes.
- *H\_N90 PPO* gene from *E. cloacae* that was codon optimized for expression in soybean, which is preceded by the promoter, leader and intron of a ubiquitin gene from *Medicago truncatula* and chloroplast targeting sequence of the *albino or pale green 6* gene (*APG6*) from *Adansonia digitata*. Following the *H\_N90 PPO* gene is the 3' untranslated region (UTR) sequence from *Zea mays*.

The first three cassettes are flanked by *loxP* excision targeting sequences. Following transformation, meristems were grown on selection media<sup>1</sup>, and plants with normal phenotype were selected based on *aadA* selectable marker in the R0 generation. After a successful transformant was selected, the *aadA* selectable marker cassette, the *cre* cassette, and SDI cassettes, were excised through Cre/*lox*-mediated auto-excision in R1 generation. These plants were self-pollinated and the R1 population was screened for the presence of T-DNA, absence of vector backbone sequences, herbicide tolerance and phenotypic assessments.

Bayer characterized the number of T-DNA inserts, the number of insert junctions, the absence of vector backbone sequences, and organization and intactness of the insert in MON 94115 soybean using whole genome sequencing (WGS). The parental cultivar, A3555, was used as the comparator in these analyses. Bayer reports a minimum average read depth of 113-fold. Bayer reports that a single copy of the T-DNA sequence was inserted into the soybean genome and that MON 94115 soybean does not contain sequences from the vector backbone. Directed DNA sequencing demonstrated that the genetic elements within the inserted T-DNA were intact, except for small deletions at the border region and the sequences removed through cre/*lox* excision when compared to vector PV-GMHT533023. The sequencing analyses also determined the nucleotide sequence for 1,000 base pairs (bp) upstream and downstream of the insertion site. A

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<sup>1</sup> The selection media contained spectinomycin for selection of transformants, and carbenicillin, cefotaxime and timentin for inhibition of *A. tumefaciens* growth.

sequence comparison between the PCR product generated from the control and the sequence generated from the 5' and 3' flanking sequence of MON 94115 soybean indicated that the T-DNA insert replaced 10 bp of soybean genomic DNA. Bayer states that these types of changes are probably the result of double stranded break repair during the *Agrobacterium*-mediated transformation process.

The stability of the inserted T-DNA sequences in MON 94115 soybean across multiple breeding generations and the comparator A3555 were evaluated by WGS followed by bioinformatics analyses. Bayer reports that the single locus of integration that was characterized in the R4 generation of MON 94115 soybean is present in all five breeding generations of the MON 94115 soybean. Within each self-crossed generation, Bayer collected genotypic segregation data to assess inheritance pattern, and these data were analyzed using Chi square analysis. Bayer concludes that the inserted DNA segregated as a single locus in accordance with the principles of Mendelian inheritance.

Bayer performed bioinformatics analyses using the nucleotide sequences obtained for the T-DNA insert and junction sequences to determine whether insertion of the introduced DNA created any potential open reading frames (ORF) that could encode for putative polypeptides. This analysis included nucleotide sequences for each of the six reading frames. Bayer evaluated the putative polypeptides against the TOX\_2024 database<sup>2</sup> to determine the similarity of these putative polypeptides to known toxins. Bayer reports that none of the putative polypeptides had significant identity with proteins in its toxin database, TOX\_2024. 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.

### Protein Safety

MON 94115 soybean was genetically engineered to express HemG-type PPO protein derived from *E. cloacae*. HemG-type PPO is a smaller PPO enzyme that catalyzes the oxidation of protoporphyrinogen IX to protoporphyrin IX, thereby conferring tolerance to PPO-inhibiting herbicides in MON 94115 soybean by maintaining the oxidation of protoporphyrinogen IX to protoporphyrin IX.

To conduct safety assessment of PPO protein, Bayer produced the PPO protein in *E. coli*. Bayer confirmed the identity and biochemical equivalence of the PPO protein expressed in *E. coli* and MON 94115 soybean, by comparing results from amino acid sequence, molecular weight and immunoreactivity, glycosylation, and functional activity.

### Protein Expression Level

Bayer quantified PPO protein levels in various tissues of MON 94115 soybean. The study included five field trial sites with four replicate plots at each site. Over-season leaf (OSL) 1-4, flower, forage, root, and grain tissue samples were collected from each replicated plot at all field sites treated with flumioxazin herbicides. Samples were prepared and analyzed using Enzyme-linked immunosorbent assay. Bayer reports that mean level of PPO protein in MON 94115 soybean across all sites was highest in OSL4 at 90

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<sup>2</sup> 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.

micrograms/gram of tissue dry weight (DW) and lowest in grain at 0.97 micrograms/gram DW.

Bayer used a weight of evidence approach to assess the safety of the PPO protein. Bayer highlights that the donor organism, *E. cloacae*, is a common soil, water, plants, and the gastrointestinal tracts bacterium that is ubiquitously present in the environment. Bayer demonstrated that the PPO protein was rapidly digested by pepsin and pancreatin and rapidly lost activity when heated at or above 55 °C. Bayer carried out bioinformatics analysis and concludes that PPO protein in MON 94115 does not share similarity with any sequences in the TOX\_2024 database. Bayer states that there was no evidence of acute toxicity in mice that were dosed orally at 5000 milligrams of PPO protein/kilogram of body weight. Taken together, Bayer concludes that dietary exposure to PPO protein in MON 94115 soybean poses no meaningful risk to animal health.

### Animal Food Use

The developer states that MON 94115 soybean is expected to be grown for the same uses as currently commercialized soybean, and no new or specialty food or feed uses are anticipated. The typical uses of soybean-derived food and feed are well documented in Organisation for Economic Co-operation and Development (OECD) soybean composition consensus document.<sup>3</sup> 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.

### Composition

#### Scope of Analysis

Bayer analyzed the nutrient composition of MON 94115 soybean and soybean variety A3555 (control) that were grown and harvested under similar conditions. Compositional analyses of grain and forage samples were reported for components listed in the OECD soybean composition consensus document.

#### Study Design

Bayer conducted field trials in 2023 at five locations in the United States. A randomized complete block design with five replicate plots was used at each field site. Forage samples were harvested at R5 growth stage and were shipped on dry ice from the field sites to Bayer. Grain was harvested at physiological maturity and shipped at ambient temperature from the field sites to Bayer. A subsample for compositional analysis was obtained from forage and grain samples and stored at approximately -20°C prior to nutrient analyses.

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<sup>3</sup> Organisation for Economic Co-operation and Development. 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. Series on the Safety of Novel Foods and Feeds No. 25. ENV/JM/MONO 24. OECD, Paris

Components that were analyzed in forage samples included crude protein, crude fat, ash, carbohydrates by calculation,<sup>4</sup> acid detergent fiber (ADF), and neutral detergent fiber (NDF). Nutrient analyses in grain included the above-mentioned components plus 18 amino acids, 22 fatty acids, two minerals, two vitamins, phytic acid, raffinose, lectin, stachyose and trypsin inhibitor, and three isoflavones. Bayer notes that nine of the fatty acids were not statistically analyzed because more than 50% of the observations fell below the lower limits of quantitation. Results were all expressed on a dry matter basis prior to statistical analyses. Moisture of forage and grain were not statistically analyzed. Bayer statistically compared each component for MON 94115 soybean with 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 94115 soybean and control. Differences between MON 94115 soybean and control were evaluated in context of variation within the control germplasm grown across multiple sites and of natural variability defined by values for soybean varieties in the Agriculture and Food Systems Institute Crop Composition Database (AFSI-CCDB)<sup>5</sup> and in the scientific literature.

### Results of Analyses

Bayer reports that there were no statistically significant differences between MON 94115 soybean and control for most of the analyzed components. Statistically significant differences between MON 94115 soybean and the control were reported for linolenic acid and calcium in the grain samples. However, Bayer notes that the mean values for all of these components were within the range of values observed in the AFSI-CCDB and scientific literature. Bayer concludes that the observed statistically significant differences between MON 94115 soybean and the control are not biologically meaningful from an animal food safety perspective.

### Summary of Compositional Analyses

Bayer states that expression of the PPO protein that imparts tolerance to different herbicides does not meaningfully alter the nutrient composition of MON 94115 soybean. Bayer concludes that these results support the conclusion that forage and grain obtained from MON 94115 soybean are compositionally equivalent to the control in the levels of key nutrients and anti-nutrients.

### Conclusion

CVM evaluated Bayer's submissions to determine whether MON 94115 soybean 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 94115 soybean and the animal foods derived from it are as safe as and are not materially different in composition or any other relevant parameter

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<sup>4</sup> Percent carbohydrates = 100% - (% protein + % fat + % moisture + % ash).

<sup>5</sup> Agriculture and Food Systems Institute (AFSI) Crop Composition Database: <https://www.cropcomposition.org>.

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 94115 soybean for use in animal food to be complete.

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