U.S. FOOD & DRUG ADMINISTRATION

Human Foods Program

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HFP Note to the File

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To: Administrative Record, BNF No. 000203

Subject: Soybean with transformation event MON 94115 (MON 94115 soybean)

Keywords: *Glycine max*, soybean, herbicide tolerance, protoporphyrinogen IX oxidase (PPO)-inhibiting herbicides, *ppo* gene (*H_N90 PPO*), PPO protein, *Enterobacter cloacae,* Bayer CropScience LP, MON 94115, OECD unique identifier MON-94115-8

Summary

Bayer CropScience LP (Bayer) has completed a consultation with the Food and Drug Administration (FDA) on food derived from MON 94115 soybean with an herbicide tolerance trait.[1] MON 94115 soybean expresses PPO encoded by the *H_N90 PPO* gene conferring tolerance to PPO-inhibiting herbicides. This document summarizes Bayer's conclusions and supporting data and information that FDA's Human Foods Program (HFP, we) evaluated pertaining to human food uses. FDA's Center for Veterinary Medicine summarizes its evaluation pertaining to animal food uses in a separate document.

Based on the safety and nutritional assessment Bayer has conducted, it is our understanding that Bayer concludes:

- it has not introduced into human food a new protein or other substance that would require premarket approval as a food additive; and
- human food from MON 94115 soybean is comparable to and as safe as human food from other soybeans.

HFP evaluated data and information supporting these conclusions and considered whether MON 94115 soybean raises other regulatory issues involving human food within FDA's authority under the Federal Food, Drug, and Cosmetic Act (FD&C Act). We have no further questions at this time about the safety, nutrition, and regulatory compliance of human food from MON 94115 soybean.

[1] The United States Environmental Protection Agency (EPA) registers pesticides (including herbicides) under the Federal Insecticide, Fungicide, and Rodenticide Act. Under the FD&C Act, EPA establishes tolerances (maximum legally permissible levels) of residues of pesticides in food.

Subject of the Consultation

Crop	Soybean (*Glycine max*)
Designation	MON 94115
Intended trait	Tolerance to PPO-inhibiting herbicides
Developer	Bayer CropScience LP
Submission received	June 30, 2025
Amendment(s) received	September 30, 2025
Intended use	General use in human food
Transformation plasmid	PV-GMHT533023
Expression cassette	A protoporphyrinogen IX oxidase ppo gene *[H_N90 PPO]* from *Enterobacter cloacae*[2]
Method for conferring genetic change	*Agrobacterium*-mediated transformation

Molecular Characterization

Confirmation of intended genetic change

Bayer developed MON 94115 soybean from meristem explants of A3555, a non-genetically engineered (non-GE) soybean cultivar. After transformation, explants were grown on media containing spectinomycin to select for lines containing T-DNA.[3] Spectinomycin-resistant lines were grown and screened using quantitative polymerase chain reaction (qPCR) to confirm the absence of *Cpf1*-gRNA, selectable marker, and Cre/*lox* associated sequences. Bayer conducted molecular, phenotypic, and herbicide tolerance assessments to select MON 94115 as the lead event.

Bayer used next generation sequencing (NGS) and PCR product sequencing approaches to assess the integrity and copy number of the inserted T-DNA in MON 94115 soybean. Through comparison of MON 94115 soybean sequences to both non-GE control soybean genomic DNA and transformation plasmid sequences, Bayer identified two junction sequences and a single insert of *H_N90 PPO* sequence within the genome of MON 94115 soybean. Bayer separately confirmed junction sequences by amplifying flanking genomic regions and directly sequencing products from overlapping PCR. Sequencing analysis confirmed that the insertion is a single and exact copy of the T-DNA from the transformation plasmid. Bayer reported that the junction sequence analysis also revealed the deletion of 10 base pairs of genomic DNA at the insertion site in MON 94115 soybean.

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[2] *H_N90 PPO* expression cassette contains a sequence encoding chloroplast transit peptide. As PPO protein matures, chloroplast transit peptides are cleaved.

[3] T-DNA initially contained sequences for the *aadA* gene, *Cpf1*-gRNA, and Cre recombinase flanked by the *loxP* recognition site. Bayer used *aadA* to select seedlings resistant to spectinomycin and *Cpf1*-gRNA to insert the T-DNA into a specific location in the soybean genome. Bayer then used Cre/*lox* recombinase to remove *aadA* and *Cpf1*-gRNA making MON 94115 soybean free of genes and sequences used in selection and precise insertion.

Absence of vector backbone DNA

Bayer used PCR and NGS sequencing approaches to confirm the absence of vector backbone sequences in the genome of MON 94115 soybean. Bayer concluded from the result of its analysis that vector backbone sequences are not present in MON 94115 soybean.

Inheritance and stability

Bayer analyzed *H_N90 PPO* sequences from the T-DNA insert in MON 94115 soybean across multiple generations to determine its pattern of inheritance and generational stability. Bayer evaluated NGS data from five generations of MON 94115 soybean to assess generational stability. Based on the NGS sequencing reads and PCR product sequencing comparison, Bayer found a single copy of the insert and an identical pair of junction sequences throughout the tested generations, confirming the genomic stability of the *H_N90 PPO* sequence. Bayer used PCR-based genotyping and chi-square analysis of three segregating generations to examine the inheritance pattern of the MON 94115 T-DNA insert. From the results, Bayer concluded that the T-DNA insert in MON 94115 soybean is inherited according to Mendelian principles of inheritance for a single locus.

Open reading frame analysis

Bayer used bioinformatic analysis to evaluate whether unintended open reading frames (ORFs) were created due to the inserted DNA in MON 94115 soybean and, if so, whether putative expression products raised toxicity or allergenicity concerns in human food. Bayer translated nucleotide sequences for potential ORFs from all six reading frames of each stop-to-stop codon segments from across soybean genomic junctions spanning the inserted T-DNA. The resulting putative polypeptide sequences were compared to the sequences of known allergens[4], toxins[5], and to proteins[6] associated with adverse health effects for humans. Bayer found no relevant sequence similarities across the length of the putative polypeptides when compared to sequences in the allergen, toxin, and protein databases.[7] The allergen sequence comparison also included identification of significant sequence similarities with >35% identity across an 80 amino acid sliding window or identical matches of eight contiguous amino acids to sequences in the allergen database. Considering the absence of transcriptional and translational regulatory elements, Bayer reported that putative peptides, if any, generated from MON 94115 insert would not share significant similarity or identity to known allergens, toxins, or other biologically active proteins that would raise food safety concerns.

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[4] Bayer's allergen sequence dataset (AD-2024) consists of allergen, gliadin, and glutenin sequences from "COMprehensive Protein Allergen REsource" (2024) database by the Health and Environmental Sciences Institute (HESI, https://comparedatabase.org).

[5] Toxin sequences were derived from the Swiss-Prot database (2024) (https://www.uniprot.org/). Bayer described their dataset (TOX-2024) as "a subset of sequences selected using a keyword search and filtered to remove likely non-toxin proteins."

[6] Bayer's protein database (PRT_2024) was derived from National Center for Biotechnology Information (2024) (NCBI, https://www.ncbi.nlm.nih.gov).

[7] Relevant sequence similarity was determined by visual inspection of the sequence alignment, calculated percent identity, and the *E*-value of ≤1x10-[5] to known allergen and toxin sequences.

Introduced Protein: Protoporphyrinogen IX oxidase (PPO)

Intended trait	Tolerance to PPO-inhibiting herbicides
Source organism	*Enterobacter cloacae*
Protein description	The introduced PPO is a HemG isoform of PPO enzyme class and is identical to PPO from *E. cloacae*, except for the addition of a chloroplast targeting sequence.[8]
Intended function	PPO oxidizes protoporphyrinogen IX to protoporphyrin IX, an important step in the biosynthesis of tetrapyrroles such as chlorophyll and heme. Introduction of PPO maintains the oxidation reaction needed for the continued functioning of chlorophyll biosynthetic pathway, making plants tolerant to PPO-inhibiting herbicides.

Bayer used enzyme linked immunosorbent assay to measure the concentration of PPO in MON 94115 soybean seed. Bayer reports that soybean seeds contain the lowest amount of PPO at 0.97 µg/g dry weight compared to other plant parts.

Bayer used multiple evidence-based approaches to assess the safety of PPO in MON 94115 soybean. Bayer reports that the bacteria harboring identical PPO sequences are ubiquitously present in the environment, including gastrointestinal tract of humans and animals. Although these bacteria are known to cause opportunistic infections, this class of enzyme is present in different forms of life including bacteria, cyanobacteria, algae, humans, animals, and plants. Bayer also reports that homologues of *H_N90 PPO* are found in probiotic bacteria, which humans consume on a regular basis.

Bayer conducted bioinformatics analysis to assess the toxicity and allergenicity of the PPO protein. Bayer compared the amino acid sequences of the PPO to the sequences of known allergens[4] and toxins[5]. None of the sequences matched to PPO with significant similarity above the set cutoff value,[7] indicating that PPO is not structurally similar to any of the compared toxins or allergens. The allergen sequence comparison also included identification of significant similarities with >35% identity across an 80 amino acid sliding window and identical matches of eight contiguous amino acids to sequences in the allergen database. Bayer concluded that the results from the bioinformatics comparison confirmed the absence of relevant amino acid sequence similarity between PPO and toxins or allergens.

Bayer conducted *in vitro* digestive fate study to assess the digestibility of the introduced PPO protein. Bayer first obtained PPO protein by expressing it in *E. coli* and confirmed its identity, equivalence, and glycosylation status compared to PPO expressed in MON 94115 soybean. Bayer then treated the microbially expressed PPO with the digestive enzymes, pepsin and pancreatin. Results showed that pepsin degraded at least 97.5% of intact PPO within 30 seconds and pancreatin degraded approximately 97.5% of the PPO within 5 minutes, confirming the degradation of PPO protein by these digestive enzymes.

Bayer assessed integrity and functionality of PPO at different elevated temperatures. For this study, PPO was treated with temperature ranging from 25 to 95 ^{o}C. Integrity was determined by measuring the

[8] Larue, C.T., J.E. Ream, X. Zhou, F. Moshiri, A. Howe, M. Goley, O.C. Sparks, S.T. Voss, E. Hall, C. Ellis, J. Weihe, Q. Qi, D. Ribeiro, X. Wei, S. Guo, A.G. Evdokimov, M.J. Varagona and J.K. Roberts. 2020. Microbial HemG-type protoporphyrinogen IX oxidase enzymes for biotechnology applications in plant herbicide tolerance traits. Pest Management Science 76:1031-1038.

intensity of the PPO band in SDS page gel, and functionality was determined by the ability of PPO to convert protoporphyrinogen IX to protoporphyrin IX by enzyme assay. Bayer reported that while heat treatment slightly reduced band intensity of PPO in SDS-PAGE gel, enzymatic activity stopped relative to the unheated control when treated above 55 °C for 15 or 30 minutes. Thus, while the integrity of the PPO enzyme did not change substantially, its functionality decreased as temperature increased. Bayer concluded that heat treatment during standard processing of soybean-containing foods will degrade PPO protein functionality, and that PPO protein would consequently not be consumed as an active protein in human food products derived from MON 94115 soybean.

Bayer conducted acute oral toxicity studies in mice to evaluate the potential toxicity of the PPO protein. Bayer reported the results of the acute oral toxicity study and stated that there was no evidence of adverse effects at intake levels of up to 5,000 mg PPO protein/kg body weight. Bayer concluded that the No-Observed-Adverse-Effect-Level for PPO protein is greater than the anticipated human exposure level of PPO when consumed in food.

Bayer concluded that based on the weight of the evidence, dietary exposure to PPO from MON 94115 soybean poses no meaningful risk to human health.

Human Food Nutritional Assessment

Analysis of key nutrients, anti-nutrients, and toxicants

The intended herbicide tolerant trait in MON 94115 soybean is not expected to alter levels of key nutrients, anti-nutrients, or toxicants. To assess potential unintended changes in composition relevant to safety or nutrition, Bayer conducted field trials at multiple locations across United States in 2023. MON 94115 soybean was grown along with the conventional non-GE soybean variety A3555, which has a similar genetic background (control). Mature seeds from MON 94115 soybean and the control were collected and measured for proximates (crude protein, total fat, ash), carbohydrates by calculation, fiber (acid detergent fiber and neutral detergent fiber), amino acids, fatty acids, minerals (calcium and phosphorus), vitamins (E and K1), secondary metabolites of the isoflavone class (daidzein, genistein, and glycitein) and anti-nutrients (phytic acid, raffinose, soybean lectin, stachyose, and trypsin inhibitor). MON 94115 soybean values were compared to those of the control and to publicly available soybean composition data. Bayer stated that some fatty acids (caprylic acid, capric acid, lauric acid, myristoleic acid, pentadecanoic acid, pentadecenoic acid, gamma linolenic acid, eicosatrienoic acid, and arachidonic acid) in grain with values below the level of quantitation were excluded from the statistical analysis. Among the statistically analyzed components, the mean values of linolenic acid and calcium were statistically different between MON 94115 soybean and the control. The difference in mean values between MON 94115 soybean and the control, however, were less than the observed ranges from the control measured across the experimental sites. Moreover, mean values of analytes measured from MON 94115 soybean were within the range of natural variation observed for soybean varieties reported in the published scientific literature and in AFSI CCDB[9], and therefore likely not a food safety concern.

Bayer concludes that seed from MON 94115 soybean is compositionally equivalent to the control variety in levels of key nutrients and anti-nutrients, and that its levels are within the natural variation for soybeans with respect to food safety.

[9] Agriculture and Food System Institute (AFSI) Crop Composition Database; https://www.cropcomposition.org

Conclusion

Based on the information provided by Bayer and other information available to HFP, we have no further questions at this time about the safety, nutrition, and regulatory compliance of human foods derived from MON 94115 soybean. We consider the consultation with Bayer on MON 94115 soybean to be complete.

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