U.S. FOOD & DRUG ADMINISTRATION

Human Foods Program

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HFP Note to the File

From: Jianmei Zhu,

To: Administrative Record, BNF No. 000208

Subject: Corn with transformation event MON 80616 (MON 80616 corn)

Keywords: *Zea mays* L., maize, corn, herbicide tolerance, protoporphyrinogen IX oxidase (PPO)-inhibiting herbicides, *ppo* gene (*H_N90 PPO*), PPO protein, *Enterobacter cloacae*, Bayer CropScience LLC, MON 80616, OECD unique identifier MON-8Ø616-9

Summary

Bayer CropScience LLC (Bayer) has completed a consultation with the Food and Drug Administration (FDA) on food derived from MON 80616 corn with an herbicide tolerance trait.[1] MON 80616 corn expresses protoporphyrinogen IX oxidase (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 80616 corn is comparable to and as safe as human food from other corn varieties.

HFP evaluated data and information supporting these conclusions and considered whether MON 80616 corn 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 80616 corn.

[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	Corn (*Zea mays* L.)
Designation	MON 80616
Intended trait	Tolerance to PPO-inhibiting herbicides
Developer	Bayer CropScience LLC
Submission received	January 20, 2026
Intended use	General use in human food
Transformation plasmid	PV-ZMHT530724
Expression cassette	A protoporphyrinogen IX oxidase (*ppo*) gene *H_N90 PPO* from *Enterobacter cloacae* encoding the protoporphyrinogen oxidase (PPO) protein.
Method for conferring genetic change	*Agrobacterium tumefaciens*-mediated transformation

Molecular Characterization

Confirmation of intended genetic change

Bayer developed MON 80616 corn from mature seed embryo explants of inbred line HCL301, a non-genetically engineered (non-GE) corn variety. After transformation, explants were grown on media containing glyphosate to select for lines containing T-DNA. The T-DNA contains the *H_N90 PPO* expression cassette, the *cp4 epsps* selectable marker and site-directed integration (SDI) cassettes where *cp4 epsps* selectable marker and SDI cassettes are flanked by lox sites to allow excision via the Cre recombinase. The SDI enables targeted integration of the T-DNA at a specific genome location. Selected lines were screened using quantitative polymerase chain reaction (qPCR) to confirm the absence of *cp4 epsps* selectable marker, SDI cassette, and Cre/*lox*-associated sequences. Bayer conducted molecular, phenotypic, and herbicide tolerance assessments to select MON 80616 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 80616 corn. Through comparison of MON 80616 sequences to both non-GE control corn 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 80616 corn. 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 19 base pairs of genomic DNA at the insertion site in MON 80616 corn.

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 80616 corn. Bayer concluded from the results of its analysis that vector backbone sequences are not present in MON 80616 corn.

Inheritance and stability

Bayer analyzed *H_N90 PPO* sequences from the T-DNA insert in MON 80616 corn across multiple generations to determine its pattern of inheritance and generational stability. Bayer evaluated NGS data from five generations of MON 80616 corn 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 data from three segregating generations to examine the inheritance pattern of the MON 80616 T-DNA insert. Bayer concluded that the T-DNA insert in MON 80616 corn 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 80616 corn and, if so, whether putative expression products raised toxicity or allergenicity concerns in human food. Bayer translated nucleotide sequences of potential ORFs from all six reading frames of each stop-to-stop codon segment from across corn genomic junctions spanning the inserted T-DNA. The resulting putative polypeptide sequences were compared to the sequences of known allergens,[2] toxins,[3] and to proteins[4] 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. 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 80616 insert would not share significant similarity or identity to known allergens, toxins, or other biologically active proteins that would raise food safety concerns.

Introduced Protein: Protoporphyrinogen IX Oxidase (PPO)

Intended trait	Tolerance to PPO-inhibiting herbicides
Source organism	*Enterobacter cloacae*
Protein description	The PPO is a HemG isoform of the PPO enzyme class
Intended function	PPO catalyzes the oxidation of protoporphyrinogen IX to protoporphyrin IX with molecular oxygen, 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 the chlorophyll biosynthetic pathway, making plants tolerant to PPO-inhibiting herbicides.

[2] Bayer's allergen sequence database (AD_2025) was obtained from "COMprehensive Protein Allergen REsource" (COMPARE) database from the Health and Environmental Sciences Institute (HESI, https://comparedatabase.org/), downloaded January 28, 2025, containing 2,836 sequences.
[3] Bayer's toxin protein database (TOX_2025) is a subset of sequences derived from the Swiss-Prot database (https://www.uniprot.org/) that were selected using a keyword search.
[4] Bayer's protein database (PRT_2025) was downloaded from National Center for Biotechnology Information (2025) (NCBI, https://www.ncbi.nlm.nih.gov).

Bayer used enzyme-linked immunosorbent assay (ELISA) to measure the concentration of PPO in MON 80616 corn tissues. Bayer reports that corn grain contains the lowest amount of PPO at 0.44 µg/g dry weight compared to other plant parts.

Bayer used multiple evidence-based approaches to assess the safety of PPO in MON 80616 corn. Bayer reports that the bacteria harboring identical PPO sequences are ubiquitously present in the environment, including the 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 following guidance from the Codex Alimentarius Commission.[5] Bayer compared the amino acid sequences of the PPO to the sequences of known allergens[2] and toxins[3] and concluded that the PPO protein sequence does not share meaningful similarity with sequences in the allergen or toxin databases. 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 analysis confirmed the absence of relevant amino acid sequence similarity between PPO and allergens or toxins known to have adverse effects on human health.

Bayer conducted an *in-vitro* digestive fate study to assess the digestibility of the PPO protein. Bayer first obtained PPO protein by expressing it in *E. coli* and confirmed its identity, equivalence, and glycosylation status compared to PPO protein expressed in MON 80616 corn. Bayer then treated the *E. coli*-produced 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 more than 97.5% of the PPO within 5 minutes, confirming the rapid degradation of PPO protein by these digestive enzymes.

Bayer assessed the integrity and functionality of PPO at different elevated temperatures. For this study, PPO was treated with temperatures ranging from 25°C to 95°C. Integrity was determined by measuring the 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 at higher temperatures, enzymatic activity was not detected when treated at 55°C or above for 15 or 30 minutes. Thus, while the integrity of the PPO enzyme did not change substantially at lower temperatures, its functionality decreased as temperature increased. Bayer concluded that heat treatment during standard processing of corn-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 80616 corn.

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 the tested intake level of 5,000 mg PPO protein/kg body weight. Bayer concluded that the No-Observed-Adverse-Effect-Level (NOAEL) for PPO protein is greater than or equal to 5,000 mg/kg body weight, which 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 80616 corn poses no meaningful risk to human health.

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[5] Codex Alimentarius. 2009. Foods derived from modern biotechnology. Second Edition. Codex Alimentarius Commission, Joint FAO/WHO Food Standards Programme, Food and Agriculture Organization of the United Nations, Rome, Italy.

Human Food Nutritional Assessment

The intended herbicide tolerant trait in MON 80616 corn 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 five locations across the United States in 2024. MON 80616 corn was grown along with the conventional non-GE corn variety HCL301 (control), which has a similar genetic background. Mature grain and forage from MON 80616 corn and the control were collected and measured for proximates (crude protein, total fat, ash), carbohydrates by calculation, fiber (acid detergent fiber, neutral detergent fiber, and total dietary fiber), amino acids, fatty acids, nine minerals, seven vitamins, secondary metabolites (ferulic acid, furfural, and p-coumaric acid), and anti-nutrients (phytic acid and raffinose). Forage samples were assessed for proximates, carbohydrates by calculation, fiber, and minerals.

Bayer stated that 13 fatty acids and furfural, with more than 50% of observations below the assay LOQ, were excluded from statistical analysis. Among the statistically analyzed components, the mean values of five components (protein, palmitic acid, stearic acid, arachidic acid, and potassium) in grain were statistically different between MON 80616 corn and the control. The differences in mean values between MON 80616 corn and the control, however, were within the ranges observed in the published scientific literature and in the AFSI Crop Composition Database,[6] and therefore are unlikely to be a food safety concern. Bayer concludes that grain and forage from MON 80616 corn 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 corn with respect to food safety.

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 food from MON 80616 corn. We consider the consultation with Bayer on MON 80616 corn to be complete.

JIANMEI ZHU -S

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Jianmei Zhu, Ph.D.

[6] Agriculture and Food System Institute (AFSI) Crop Composition Database; https://www.cropcomposition.org2.9