U.S. FOOD & DRUG ADMINISTRATION

Human Foods Program

Biotechnology Notification File No. 000204
HFP Note to the File

Date: February 12, 2026

From: Charles Kanobe, Ph.D., Biologist

To: Administrative Record, BNF No. 000204

Subject: Cotton with transformation event MON 96012 (MON 96012 Cotton)

Keywords: Cotton, *Gossypium hirsutum* (L.), herbicide tolerance, glyphosate, CP4 5-enolpyruvylshikimate-3-phosphate synthase, CP4 EPSPS, *cp4 epsps* gene, *Agrobacterium* sp. strain CP4, glufosinate, phosphinothricin-N-acetyltransferase, PAT, *pat* gene, *Streptomyces viridochromogenes*, dicamba, dicamba mono-oxygenase, DMO, *dmo* gene, *Stenotrophomonas maltophilia*, mesotrione, triketone dioxygenase, TDO, *TDO* gene, *Oryza sativa*, PPO-inhibiting herbicides, protoporphyrinogen IX oxidase, PPO, *ppo* gene (*H_N90 PPO*), *Enterobacter cloacae*, Bayer CropScience LP, MON 96012, OECD unique identifier MON-96Ø12-6

Summary

Bayer CropScience LP (Bayer) has completed a consultation with the Food and Drug Administration (FDA) on food derived from MON 96012 cotton with multiple herbicide tolerance traits.[1] 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 of MON 96012 cotton. FDA's Center for Veterinary Medicine summarizes its evaluation pertaining to animal food uses of MON 96012 cotton 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;
- human food from MON 96012 cotton is comparable to and as safe as human food from conventional cotton varieties.

HFP evaluated data and information supporting these conclusions and considered whether MON 96012 cotton raises other regulatory issues involving human food within FDA's authority under the Federal Food, Drug, and Cosmetic Act. We have no further questions at this time about the safety, nutrition, and regulatory compliance of human food from MON 96012 cotton.

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

Subject of the Consultation

Crop	Cotton (*Gossypium hirsutum)*
Designation	MON 96012
Intended trait 1	Tolerance to glyphosate herbicides
Intended trait 2	Tolerance to glufosinate-ammonium herbicides
Intended trait 3	Tolerance to dicamba herbicide
Intended trait 4	Tolerance to mesotrione herbicide
Intended trait 5	Tolerance to PPO-inhibiting herbicides
Developer	Bayer CropScience LP
Submission received	July 2, 2025
Amendment(s) received	January 7, 2026
Intended use	General use in human food
Transformation plasmid	PV-GHHT529207
Expression cassette 1	The *cp4 EPSPS* cassette contains a codon-optimized *aroA* gene from *Agrobacterium sp*. strain CP4, encoding the 5-enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS) protein.
Expression cassette 2	The *pat* cassette contains a codon-optimized *pat* gene from *Streptomyces viridochromogenes* encoding the phosphinothricin-N-acetyltransferase (PAT) protein.
Expression cassette 3	The *dmo* expression cassette contains a codon-optimized demethylase (*dmo*) gene from *Stenotrophomonas maltophilia* encoding the dicamba mono-oxygenase (DMO) protein.
Expression cassette 4	The *TDO* cassette contains a codon-optimized *TDO* gene from *Oryza sativa* encoding the triketone dioxygenase (TDO) protein.
Expression cassette 5	The *ppo* (*H_N90 PPO)* expression cassette contains the *H_N90 PPO* gene from *Enterobacter cloacae* encoding the protoporphyrinogen oxidase (PPO) protein.
Expression cassette 5[2]	The *aadA* gene cassette from *E. coli* transposon Tn7 encodes the 3"(9)-*O*-nucleotidyltransferase enzyme conferring resistance to spectinomycin and streptomycin. *aadA* was used as a selectable marker.
Method for conferring genetic change	*Agrobacterium tumefaciens*-mediated transformation

[2] The *aadA* gene cassette (selection marker) was removed from the MON 96012 cotton genome using the Cre/*lox* recombinase auto-excision system.

Molecular Characterization

Confirmation of intended genetic change

Bayer developed MON 96012 cotton through *Agrobacterium tumefaciens*-mediated transformation of conventional cotton variety DP393 meristem tissues. The plasmid vector PV-GHHT529207 containing T-DNA with five gene expression cassettes was used. After transformation, regenerants were rooted, and plants showing normal phenotype were selected for molecular characterization. Bayer used whole genome sequencing and bioinformatics analysis to confirm the presence of the intended T-DNA, the integrity, and copy number of the T-DNA insertion. From the results, Bayer concluded that MON 96012 cotton contained a single intact copy of the T-DNA insert. Bayer also reported that there was no DNA rearrangement at the MON 96012 cotton insertion site, although one hundred and eight (108) base pairs of genomic DNA were deleted during the integration of the T-DNA.

Absence of vector backbone DNA

Bayer analyzed next generation sequencing (NGS) data from MON 96012 cotton by aligning MON 96012 sequences with those of the transformation plasmid to confirm absence of vector backbone. From the analysis, Bayer reported that a single unpaired read mapped to the transformation plasmid backbone aligning with a few bases of an element sequence used for DNA cloning. Bayer explained that sporadic low-level detection of plasmid sequences, as described above, has previously been reported and is likely due to the presence of environmental bacteria in tissue samples used in preparation of DNA for library construction. Bayer concluded that MON 96012 cotton does not contain plasmid backbone sequences.

Inheritance and stability

Bayer used NGS to assess the inheritance and stability of MON 96012 T-DNA over three segregating generations. Genotypic segregation data were recorded and using Chi-square analysis, the observed segregation ratios were compared to the expected segregation ratios. The results showed no statistical difference between the observed and the expected segregation ratios of MON 96012 T-DNA. Bayer therefore concluded that MON 96012 T-DNA is present at a single locus within the cotton genome and is inherited according to Mendelian principles of inheritance. Bayer also concluded that together with the molecular characterization data, these results confirm that MON 96012 cotton contains a single intact copy of the T-DNA that is stably inherited across multiple generations.

Open reading frame analysis

Bayer performed bioinformatic analyses to assess whether the putative polypeptides encoded by the six open reading frames (ORF) present in MON 96012 T-DNA insert and any putative ORFs spanning the 5' and 3' insert DNA-flanking sequence junctions had potential for allergenicity, toxicity or biological activity relevant to food safety. 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. Bayer reported that none of the putative, corresponding polypeptides from the hypothetical new ORFs of MON 96012 cotton showed significant sequence nor structural similarity (E-score $<1 \times 10^{-5}$) with the sequences of known allergens

(AD_2024 database)[3], toxins (TOX_2024 database)[4] or other biologically active proteins (PRT_2024 database)[5] that could affect human health.

Introduced Protein: 5-enolpyruvylshikimate-3- phosphate synthase (CP4 EPSPS)

Intended trait	Tolerance to glyphosate herbicides
Source organism	*Agrobacterium* sp. strain CP4
Protein description	The CP4 EPSPS protein expressed in MON 96012 cotton is derived from the full-length precursor form of the CP4 EPSPS protein from *Agrobacterium* sp. strain CP4. It is fused to a chloroplast targeting peptide from *Triticum aestivum* L.
Intended function	The CP4 EPSPS protein is functionally the same as the endogenous plant EPSPS enzymes but with a reduced affinity for glyphosate. It facilitates continued functioning of the shikimate pathway in presence of glyphosate making CP4 EPSPS expressing plants tolerant to the herbicide.

Bayer used enzyme linked immunosorbent assay (ELISA) to determine the CP4 EPSPS protein concentration in the seeds of MON 96012 cotton. The mean concentration of CP4 EPSPS protein in cottonseed was 28 µg/g dry weight.

FDA evaluated the safety of CP4 EPSPS protein in previous consultations, including BNF 000173, BNF 000126, and BNF000097, that Bayer used for reference in this consultation. Bayer states that the safety assessments of CP4 EPSPS protein in those prior consultations are applicable to the CP4 EPSPS protein expressed in MON 96012 cotton. Bayer also used published literature to explain the safety of CP4 EPSPS protein, which, together with data from the previous consultations listed above, demonstrated that CP4 EPSPS is present at low levels in cottonseed; lacks similarity to known allergens, toxins, or other biologically active proteins known to have adverse effects on humans; is degraded by the digestive enzymes pepsin and pancreatin; loses activity after heat treatment; and is not acutely toxic. Bayer therefore concluded that based on the weight of evidence, CP4 EPSPS from MON 96012 cotton poses no meaningful risk to human health.[6]

Introduced Protein: phosphinothricin-N-acetyltransferase (PAT)

Intended trait	Tolerance to glufosinate herbicide

[3] The allergen protein sequence database (AD_2024) was obtained as the “COMprehensive Protein Allergen REsource" (COMPARE) database from the Health and Environmental Sciences Institute (HESI, https://comparedatabase.org). Accessed on February 2, 2024.
[4] The UniProt toxin protein database (TOX_2024) is a subset of sequences derived from the Swiss-Prot database (found at https://www.uniprot.org/) that were selected using a keyword search and filtered to remove likely non-toxin proteins. Accessed on January 11, 2024.
[5] The GenBank protein database (PRT_2024) was downloaded from NCBI on January 10, 2024.
[6] CP4 EPSPS is not used as a PIP inert ingredient in MON 96012 cotton. However, Bayer explains that its safety is supported by an EPA exemption from the requirement of a tolerance for CP4 EPSPS in all food commodities when used as a PIP inert ingredient per 40 CFR 174.523.

Source organism	*Streptomyces viridochromogenes*
Protein description	The PAT (*pat*) protein expressed in MON 96012 cotton is identical to the wild-type PAT (*pat*) from *Streptomyces viridochromogenes*, except for the lack of the first methionine removed during co-translation processing in MON 96012 cotton.
Intended function	PAT (*pat*) acetylates glufosinate to a non-herbicidal N-acetyl glufosinate, making PAT (*pat*) expressing plants tolerant to glufosinate herbicide.

Bayer used enzyme linked immunosorbent assay (ELISA) to determine the PAT (*pat*) protein concentration in MON 96012 cottonseed. The mean concentration of PAT (*pat*) protein in cottonseed was less than the limit of quantitation (LOQ) of 0.312 µg/g dry weight.

FDA evaluated the PAT (*pat*) protein present in MON 96012 cotton in previous consultations, including BNF 000135, BNF 000148, and BNF 000173, that Bayer used for reference in this consultation. Bayer states that the safety assessments of PAT (*pat)* protein in those prior consultations are applicable to the PAT (*pat)* protein expressed in MON 96012 cotton. Bayer also used published literature to explain the safety of PAT (*pat*) protein, which, together with data from the previous consultations listed above, demonstrated that PAT (*pat*) is present at low levels in cottonseed; lacks similarity to known allergens, toxins, or other biologically active proteins known to have adverse effects on humans; is degraded by the digestive enzymes pepsin and pancreatin; loses activity after heat treatment; and is not acutely toxic. Bayer therefore concludes that based on the weight of evidence, PAT (*pat*) from MON 96012 cotton poses no meaningful risk to human health.[7]

Introduced Protein: dicamba mono-oxygenase (DMO)

Intended trait	Tolerance to dicamba herbicide
Source organism	*Stenotrophomonas maltophilia*
Protein description	The DMO protein expressed in MON 96012 cotton is derived from the full-length precursor form of DMO from *Stenotrophomonas maltophilia*. It is fused to a chloroplast targeting peptide from *Arabidopsis thaliana.*
Intended function	DMO catalyzes the demethylation of dicamba to the non-herbicidal compounds 3,6-dichlorosalicylic acid and formaldehyde, making DMO expressing plants tolerant to dicamba herbicide.

Bayer used enzyme linked immunosorbent assay (ELISA) to determine the DMO protein concentration in the seeds of MON 96012 cotton. The mean concentration of DMO protein in cottonseed was 25 µg/g dry weight.

FDA evaluated the DMO protein present in MON 96012 cotton in previous consultations, including BNF 000125, BNF 000135, BNF 000148, BNF 000173, and BNF 000193, that Bayer used for reference in this consultation. Bayer states that the safety assessments of DMO protein in those prior consultations are applicable to the DMO protein expressed in MON 96012 cotton. Bayer used published literature to further explain the safety of DMO protein, which, together with data from the previous consultations listed above,

[7] PAT (*pat*) is not used as a plant-incorporated protectant (PIP) inert ingredient in MON 96012 cotton. However, Bayer explains that its safety is supported by an EPA exemption from the requirement of a tolerance for PAT in all food commodities when used as a PIP inert ingredient under 40 CFR 174.522.

demonstrated that DMO protein is present at low levels in cottonseed; lacks similarity to known allergens, toxins, or other biologically active proteins known to have adverse effects on humans; is degraded by the digestive enzymes pepsin and pancreatin; loses activity after heat treatment; and is not acutely toxic. Bayer therefore concluded that based on the weight of evidence, DMO protein from MON 96012 cotton poses no meaningful risk to human health.

Introduced Protein: Triketone dioxygenase (TDO)

Intended trait	Tolerance to mesotrione herbicide
Source organism	*Oryza sativa*
Protein description	The TDO protein expressed in MON 96012 cotton is derived from the full-length precursor form of TDO gene from *Oryza sativa*.
Intended function	TDO catalyzes the sequential double oxidation of mesotrione molecules to hydroxy-mesotrione and oxy-mesotrione, resulting in TDO expressing plants being tolerant to mesotrione herbicides.

Bayer used enzyme linked immunosorbent assay (ELISA) to determine the TDO protein concentration in the seeds of MON 96012 cotton. The mean concentration of TDO protein in cottonseed was 0.51 µg/g dry weight.

FDA evaluated the TDO protein present in MON 96012 cotton in a previous consultation, BNF 000193, that Bayer used for reference in this consultation. Bayer states that the safety assessments of TDO protein in that prior consultation are applicable to the TDO protein expressed in MON 96012 cotton.
Bayer also used published literature to explain the safety of TDO protein, which, together with the data from BNF 000193, demonstrated that TDO is present at low levels in cottonseed; lacks similarity to known allergens, toxins, or other biologically active proteins known to have adverse effects on humans; is degraded by the digestive enzymes pepsin and pancreatin; loses activity after heat treatment; and is not acutely toxic. Bayer therefore concluded that based on the weight of evidence, TDO from MON 96012 cotton poses no meaningful risk to human health.

Introduced Protein: Protoporphyrinogen IX oxidase (PPO)

Intended trait	Tolerance to PPO-inhibiting herbicides
Source organism	*Enterobacter cloacae*
Protein description	The PPO protein expressed in MON 96012 cotton is derived from the full-length precursor form of PPO gene from *Enterobacter cloacae*.
Intended function	PPO catalyzes the continued oxidation of protoporphyrinogen IX to protoporphyrin IX with molecular oxygen, maintaining the production of heme and chlorophyll in presence of PPO-inhibiting herbicides, making expressing plants tolerant to PPO-inhibiting herbicides.

Bayer used enzyme linked immunosorbent assay (ELISA) to determine the PPO protein concentration in the seeds of MON 96012 cotton. The mean concentration of PPO protein in cottonseed was 0.37 µg/g dry weight.

Bayer conducted bioinformatics analysis to assess the toxicity and allergenicity of the PPO protein. Bayer compared the amino acid sequence of the PPO protein to the sequences of known allergens[8] and toxins[9], associated with adverse health effects for humans to identify significant sequence similarities (E score $\leq 1x10^{-5}$) across the length of the proteins. The allergen sequence comparison also included identification of significant similarities with >35% identity across an 80 amino acid sliding window or identical matches of eight contiguous amino acid to sequences in the allergen database. Bayer concluded that the results from the bioinformatics comparison showed absence of relevant amino acid sequence similarity between PPO and allergens or toxins known to have adverse effects on human health.

Bayer conducted *in vitro* digestibility and heat sensitivity studies to further assess the safety of the introduced PPO protein. Bayer treated microbially-produced and purified PPO with the digestive enzymes, pepsin and pancreatin. Analysis of the data showed that 98.7% of intact PPO was degraded within 30 seconds of exposure to pepsin, while 97.5% of intact PPO was degraded within 5 minutes of exposure to pancreatin. Bayer indicated that the rapid degradation of the intact PPO protein by pepsin and pancreatin indicates that the protein is unlikely to pose any safety concern to human health.

To test heat stability of PPO protein, Bayer used *E. coli*-produced and purified PPO exposed to different temperatures, ranging from 25°C to 95°C. Bayer then examined the impact of temperature on enzymatic activity and protein integrity. Bayer reported that PPO retained functional activity when incubated at 25°C and 35°C for 15 and 30 minutes. However, incubation at 35°C for 15 and 30 minutes reduced functional activity of the protein to 61% and 29%, respectively. Bayer reported that incubation of PPO protein at 55°C or higher for 15 and 30 minutes resulted in complete loss of functional activity of the protein. In addition, Bayer reported that incubation of PPO protein at 25, 37, and 55°C for 15 and 30 minutes did not result in loss of protein band intensity, although at 55°C, there were slight aggregation products compared to the control. Incubation at 75 and 95°C resulted in a slight decrease in the intensity of the PPO protein band with appearance of few aggregation bands. Bayer explained that taken together, these results demonstrate that temperature has a considerable effect on PPO activity and that the protein behaves with a predictable tendency to lose functional activity at temperatures 55°C or greater.

Bayer conducted an acute oral toxicity study in mice to evaluate the potential toxicity of the PPO protein. From the results, Bayer reported that there was no evidence of adverse effects at intake levels of up to 5,000 mg/kg body weight (bw), the highest dose tested. Bayer therefore concluded that the No-Observed-Adverse-Effect-Level (NOAEL) for the PPO protein is greater than or equal to 5000 mg/kg bw., the highest intake level tested.

Based on the weight of evidence presented above, Bayer concluded that dietary exposure to PPO protein from MON 96012 cotton poses no meaningful risk to human health.

Human Food Nutritional Assessment

The intended traits in MON 96012 cotton are not expected to alter levels of key nutrients or antinutrients. To assess potential unintended changes in composition relevant to safety or nutrition, Bayer analyzed cottonseeds from MON 96012 cotton and a non-genetically engineered (non-GE) control grown at five

[8] The allergen protein sequence database (AD_2024) was obtained as the "COMprehensive Protein Allergen REsource" (COMPARE) database from the Health and Environmental Sciences Institute (HESI, https://comparedatabase.org).

[9] The toxin database (TOX_2024) was a subset of sequences derived from the Swiss-Prot database (found at https://www.uniprot.org/) that were selected using a keyword search.

locations in the United States in 2023. In their analyses, Bayer followed considerations as defined by the OECD in their revised consensus document on compositional considerations for new varieties of cotton.[10] Cottonseed samples were assessed for moisture[11] and levels of nutrients including proximates (protein, total fat and ash), amino acids, fatty acids, carbohydrates by calculation, fiber (acid detergent fiber (ADF), neutral detergent fiber (NDF) and total dietary fiber (TDF)), minerals and vitamins. Cottonseed samples were also assessed for levels of anti-nutrients including dihydrosterculic acid, free gossypol, malvalic acid, sterculic acid, and total gossypol. Bayer compared mean values from MON 96012 cotton to those obtained from the non-GE control. The results were also evaluated in the context of natural variation including comparison to ranges from publicly available literature and the Agriculture and Food Systems Institute (AFSI) crop composition database (CCDB).[12] Comparison of results to literature ranges provides context for natural variation of plant composition resulting from a combination of genetic diversity and environmental conditions at time of production.

Bayer reported the results of its analysis, noting that there were no statistically significant differences observed between MON 96012 cotton and the non-GE control for 29 of the cottonseed-derived analytes. Eleven (11) MON 96012 cotton analytes (arginine, total fat, myristic acid, palmitic acid, palmitoleic acid, stearic acid, oleic acid, linolenic acid, phosphorus, and dihydrosterculic acid) were statistically different when compared to the non-GE control. However, for these eleven analytes, the mean values of MON 96012 cotton were within the ranges observed in the literature and/or the AFSI-CCDB. Bayer explained that these results indicate that MON 96012 was not a major contributor of compositional variation in the cottonseed and that the observed statistical differences were therefore not biologically meaningful from a food safety perspective. Bayer therefore concluded that the results of the nutrient composition assessment demonstrate that cottonseed derived from MON 96012 cotton is compositionally comparable to that of non-GE control cotton and other cotton varieties with a history of safe use in food.

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 96012 cotton. We consider the consultation with Bayer on MON 96012 cotton to be complete.

CHARLES KANOBE -S

Digitally signed by CHARLES KANOBE -S
Date: 2026.02.12 09:19:29 -05'00'

Charles Kanobe, Ph.D.

[10] Consensus document on the compositional considerations for new varieties of cotton (*Gossypium hirsutum* and *Gossypium barbadense*): Key food and feed nutrients and anti-nutrients. ENV/JM/MONO(2004)16. Organisation for Economic Co-operation and Development, Paris, France.

[11] Moisture values were used for conversion of components from fresh weight to dry weight only and hence were not statistically analyzed.

[12] AFSI ranges were from AFSI CCDB, 2024.