Biotechnology Notification File No. 000195

HFP Note to the File

Date: March 25, 2025

From: Arati N Poudel, Ph.D.

To: Administrative Record, BNF No. 000195

Subject: Corn with transformation event MON 94804 (MON 94804 corn)

Keywords: Corn, maize, *Zea mays*, short stature corn, *ZmGA20ox3* gene, *ZmGA20ox5* gene, gibberellic acid (GA) hormone, GA20ox_SUP miRNA, RNA interference (RNAi), *Agrobacterium tumefaciens*, Bayer CropScience LP, MON 94804 corn, OECD Unique Identifier MON-948Ø4-4.

Summary

Bayer CropScience LP (Bayer) has completed a consultation with the Food and Drug Administration (FDA) on food derived from MON 94804 corn. MON 94804 corn was engineered to express GA20ox_SUP miRNA, thereby suppressing expression of the plant hormone gibberellic acid (GA) biosynthetic genes *ZmGA20ox3* and *ZmGA20ox5*. Suppression of these biosynthetic genes leads to reduced levels of GA and shorter internode length, resulting in a short stature phenotype. This document summarizes Bayer's conclusions and supporting data and information that FDA's Human Food 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 94804 corn is comparable to and as safe as human food from other corn, or comparable subset of corn varieties.

HFP evaluated data and information supporting these conclusions and considered whether MON 94804 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 94804 corn.

The U.S. Environmental Protection Agency (EPA) evaluates and authorizes the use of plant-incorporated protectants (PIPs) under the FD&C Act and the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). A PIP is defined in 40 CFR 174.3 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." The term pesticide, as defined in FIFRA section 2(u) includes "any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest; any substance or mixture of substances intended for use as a plant regulator, defoliant, or desiccant; and any nitrogen stabilizer."

Subject of the Consultation

Crop	Corn
Designation	MON 94804
Intended trait	Short stature corn
Developer	Bayer CropScience LP
Submission received	February 3, 2023
Amendment(s) received	February 17 and February 21, 2023; May 30, August 7, and October 10, 2024
Intended use	General use in human food
Transformation plasmid	PV-ZMAP527892
Expression cassette 1	The *GA20ox-SUP* suppression cassette contains the inverted repeat nucleotide sequence of *ZmGA20ox3* and *ZmGA20ox5* genes from corn, encoding *GA20ox_SUP miRNA*, the expression of which is intended to suppress GA hormone in plant internodes. Transcription of the miRNA is regulated by a promoter from rice tungro bacilliform virus (RTBV), having high expression in internodes and lower expression in reproductive tissues.
Method for conferring genetic change	*Agrobacterium*-mediated transformation

Molecular Characterization

Confirmation of intended genetic change

Bayer developed MON 94804 corn from mature embryo explants of corn (*Zea mays*) inbred line HCL301. After transformation, explants were grown on media containing glyphosate to select for successful transformants.[1] The glyphosate tolerant lines that are positive for the T-DNA and negative for the vector backbone were identified using polymerase chain reaction (PCR). Bayer removed the selectable marker from the lead events[2] and selected MON 94804 as its commercial event.

Following transformation and selection of the event MON 94804, Bayer used next generation sequencing (NGS) and PCR product sequencing approaches to assess the integrity and copy number of the inserted

[1] The T-DNA in the PV-ZMAP527892 plasmid contains a *cp4 epsps* selectable marker cassette. Expression of CP4 EPSPS confers tolerance to glyphosate herbicide.

[2] The *cp4 epsps* selectable marker cassette is flanked by *loxP* excision targeting sequences. Following the selection of transformants, Bayer crossed these with a corn line expressing Cre recombinase. Bayer then used Cre/*lox*-mediated recombination to remove the selectable marker cassette. Bayer confirmed the absence of the selectable marker and *cre* gene by PCR sequencing and NGS analysis.

T-DNA. Through comparison of MON 94804 corn DNA sequences to both non-genetically engineered (GE) control corn genomic DNA and transformation plasmid sequences, Bayer identified two junction sequences and a single insertion of T-DNA. Bayer separately analyzed the junction sequences by amplifying insert and flanking genomic regions and directly sequencing the PCR products. Sequencing analysis confirmed that the insert 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 41 base pairs of genomic DNA at the insertion site in MON 94804 corn.

Absence of vector backbone DNA

Bayer used PCR, and NGS sequencing approaches to confirm the absence of vector backbone sequences in MON 94804 corn.[3] Bayer concluded from the result of its analysis that vector backbone sequences are not present in MON 94804 corn.

Inheritance and stability

Bayer analyzed the T-DNA insert in MON 94804 corn across multiple generations to determine its pattern of inheritance and generational stability. Bayer evaluated NGS data from five generations of MON 94804 corn to assess the generational stability. Based on the NGS sequencing reads and PCR product sequencing comparison, Bayer found a single copy of the T-DNA insert and an identical pair of junction sequences throughout the tested generations, confirming the genomic stability of the inserted sequence. Bayer used PCR-based genotyping and chi-square analysis of three segregating generations to conclude that the T-DNA insert in MON 94804 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 because of the inserted DNA in MON 94804 corn and, if so, whether putative expression products raised toxicity or allergenicity concerns in human food. Bayer translated ORFs (from stop codon to stop codon, in all six reading frames) in the MON 94804 insert DNA and across flanking genomic DNA sequences. The resulting putative polypeptide sequences were compared to the sequences of known allergens[4], toxins[5], and to biologically active 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 acid 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 94804 insert would not share significant similarity or identity to known allergens, toxins, or other biologically active proteins that would raise food safety concern.

---

[3] Bayer also confirmed the absence of backbone sequence from the plasmid that Bayer used to transform and generate the Cre line.

[4] Bayer's allergen sequence dataset consists of allergen, gliadin, and glutenin sequences in "COMprehensive Protein Allergen REsource" (2024) database from 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 as "a subset of sequences selected using a keyword search and filtered to remove likely non-toxin proteins."

[6] Bayer derived protein sequences with biological activity 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-score of ≤1e-5 to known allergen and toxin sequences.

Characterization of the Intended Trait

Bayer engineered MON 94804 corn to lower the level of GA hormone through suppression of *ZmGA20ox3* and *ZmGA20ox5* genes expression using RNAi. Bayer measured expression levels of ZmGA20ox3 and ZmGA20ox5 transcripts confirming that the RNAi cassette suppressed the internode specific *ZmGA20ox3* and *ZmGA20ox5* genes but not their closest homologue *ZmGA20ox1*. The use of internode specific RTBV promoter targeted gene suppression in internode, but not in other parts of the plant. Bayer then measured and confirmed the reduction of GA hormone in internodes of MON 94804 corn, which as expected, remain unchanged in internode of non-GE control, and grain of MON 94804 corn.

Human Food Nutritional Assessment

To ensure the absence of potential unintended changes in components relevant to safety or nutrition, Bayer analyzed grain from MON 94804 corn and a non-GE hybrid line with a similar genetic background (control). These were grown under typical agronomic practices, at five sites in the United States in 2020. Bayer explained that it followed principles outlined in the Organization for Economic Co-operation and Development (OECD) consensus document for its analysis of key nutrients, anti-nutrients and secondary metabolites in corn.[8] In grain, Bayer measured proximates (protein, total fat, and ash), amino acids, fatty acids, carbohydrates by calculation, fiber (acid detergent fiber, acid detergent lignin, neutral detergent fiber and total dietary fiber), minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc), vitamins (A, B_1, B_2, B_3, B_6, B_9, and E), anti-nutrients (phytic acid and raffinose) and secondary metabolites (ferulic acid, furfural and p-coumaric acid). Bayer compared data from MON 94804 corn to the control, and to ranges from publicly available literature and databases.[9, 10] These comparisons account for the natural variation in plant composition resulting from a combination of genetic diversity and environmental conditions at the time of production.

Bayer reported that among the measured components in grain from MON 94804 corn, several components had more than 50% of the observations below the limit of quantitation and were excluded from statistical analysis. Bayer statistically compared the values of the remaining components of MON 94804 corn with the control and found that many of these components were statistically similar. However, the values of four components (stearic acid, total dietary fiber, calcium, and ferulic acid) were statistically different between MON 94804 corn and the control. For these components, Bayer explained that the mean difference in component values between MON 94804 corn and the control was less than the range of the control values, indicating that these observed differences were less than the natural variations. In addition, mean values for these components in MON 94804 corn were within the range of values observed in the literature or the publicly available databases, and therefore likely not a food safety concern. Hence, Bayer concluded that grain from MON 94804 corn is compositionally comparable in levels of key nutrients and anti-nutrients to that from other commercially available corn with a history of safe use with respect to human food safety.

---

[8] Organisation for Economic Co-operation and Development. 2002. Consensus document on compositional considerations for new varieties of maize (*Zea mays*): Key food and feed nutrients, anti-nutrients, and secondary plant metabolites. OECD ENV/JM/MONO 25. OECD, Paris, France.

[9] AFSI. 2020. Crop Composition Database, Version 8.0, Agriculture & Food Systems Institute, https://www.cropcomposition.org (Accessed January 4, 2021)

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

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 94804 corn. We consider the consultation with Bayer on MON 94804 corn to be complete.

ARATI NEPAL POUDEL -S

Digitally signed by ARATI NEPAL POUDEL -S
Date: 2025.03.25 12:23:51 -04'00'

Arati N Poudel, Ph.D.