Biotechnology Notification File No. 000173
CFSAN Note to the File

Date: June 23, 2022

From: Patrick Cournoyer, PhD

To: Administrative Record, BNF No. 000173

Subject: Corn with insertion event MON 87429 (MON 87429 corn)

Keywords: Corn; maize; *Zea mays*; herbicide tolerance, dicamba, demethylase (*dmo*) gene, dicamba mono-oxygenase, DMO, *Stenotrophomonas maltophilia*, glufosinate, *pat* gene, phosphinothricin-N-acetyltransferase, PAT, *Streptomyces viridochromogenes*, aryloxyphenoxypropionate (AOPP) acetyl coenzyme A carboxylase (ACCase) inhibitors, quizalofop, 2,4-dichlorophenoxyacetic acid (2,4-D), modified version of R-2,4-dichlorophenoxypropionate dioxygenase (*Rdpa*) gene, RdpA, FT_T, *Sphingobium herbicidovorans*, glyphosate, male sterility, *aroA* gene, CP4 5-enolpyruvylshikimate-3-phosphate synthase, CP4 EPSPS, *Agrobacterium* sp. strain CP4, Bayer CropScience LP, MON 87429, OECD Unique Identifier MON-87429-9

Summary

Bayer CropScience LP (Bayer)[1] has completed a consultation with the Food and Drug Administration (FDA) on food derived from MON 87429 corn with multiple herbicide tolerance traits and glyphosate-inducible male sterility.[2] MON 87429 corn expresses dicamba mono-oxygenase (DMO) for tolerance to dicamba herbicide; phosphinothricin-N-acetyltransferase (PAT) for tolerance to glufosinate herbicide; a modified R-2,4-dichlorophenoxyproprionate dioxygenase (RdpA) (referred to by Bayer as FT_T) for tolerance to aryloxyphenoxypropionate acetyl coenzyme A carboxylase inhibitors (so-called "FOPs" herbicides such as quizalofop) and 2,4-dichlorophenoxyacetic acid (2,4-D) herbicides; and CP4 5-enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS) for tolerance to glyphosate herbicide and for glyphosate-inducible male sterility. This document summarizes Bayer's conclusions and supporting data and information that FDA's Center for Food Safety and Applied Nutrition (CFSAN, we)

[1] Monsanto Company submitted the notice for BNF No. 000173. In a letter dated August 3, 2020, FDA was informed that Monsanto Company plant products "which were consulted on for food and feed safety and those still in the process" would be transferred to the legal entity Bayer CropScience LP, effective August 1, 2020.

[2] 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.

evaluated pertaining to human food uses. FDA's Center for Veterinary Medicine summarizes its evaluation pertaining to animal food uses in a separate document.

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 87429 corn is comparable to and as safe as human food from other corn varieties.

CFSAN evaluated data and information supporting these conclusions and considered whether MON 87429 corn 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 87429 corn.

Subject of the Consultation

Crop	Corn
Designation	MON 87429
Intended trait	Tolerance to dicamba herbicide
Intended trait	Tolerance to glufosinate herbicide
Intended trait	Tolerance to aryloxyphenoxypropionate (AOPP) acetyl coenzyme A carboxylase (ACCase) inhibitor herbicides (so called "FOPs" herbicides, e.g., quizalofop); and tolerance to some synthetic auxin herbicides, including 2,4-D
Intended trait	Tolerance to glyphosate herbicide and glyphosate-inducible male sterility
Developer	Bayer CropScience LP
Submission received	February 5, 2019
Amendments received	August 7, 2019; June 4, 2020; May 18, 2021
Intended use	General use in human food
Transformation plasmid	PV-ZMHT519224
Expression cassette 1	A demethylase (*dmo*) gene from *Stenotrophomonas maltophilia* encoding dicamba mono-oxygenase (DMO)
Expression cassette 2	Phosphinothricin-N-acetyltransferase (*pat*) gene from *Streptomyces viridochromogenes* encoding PAT
Expression cassette 3	A modified version of R-2,4-dichlorophenoxypropionate dioxygenase (*Rdpa*) gene from *Sphingobium herbicidovorans*

Expression cassette 4	*aroA* gene from the *Agrobacterium* sp. strain CP4 encoding CP4 EPSPS, with a regulatory element targeting mRNA for degradation in tassel tissues
Method for conferring genetic change	Agrobacterium-mediated transformation

Molecular Characterization

Confirmation of intended genetic change

Bayer used high throughput sequencing and junction sequence analysis to assess the integrity and copy number of the DNA insertion. Bayer identified two junction sequence classes, consistent with the presence of a single insertion. Bayer amplified the insertion and flanking genomic sequences using polymerase chain reaction (PCR) and conducted sequencing analysis. Sequencing analysis revealed that the insertion includes a single, intact copy of the T-DNA sequence. Comparison to the untransformed parental variety revealed a 54 base pair deletion of corn genomic DNA at the insertion site. Small DNA insertions (29 and 31 base pairs) were found at each junction between T-DNA and corn genomic DNA.

Absence of vector backbone DNA

Bayer analyzed high throughput sequencing data from MON 87429 corn for the presence of sequences from the vector backbone. Bayer did not detect vector backbone sequences.

Inheritance and stability

Bayer analyzed high throughput sequencing data from five generations of seed-propagated MON 87429 corn. Bayer found consistent junction sequences in each generation, confirming the stable inheritance of the insertion. Bayer observed the expected 1:1 Mendelian segregation ratio for the hemizygous insertion using PCR genotyping and chi-square analysis.

Open reading frame analysis

Bayer identified new open reading frames formed at the junctions between inserted DNA and corn genomic DNA in all six reading frames from stop codon to stop codon. In the unlikely event the putative polypeptides were to occur in food, Bayer found that putative polypeptides over eight amino acids in length lacked relevant sequence similarity[3] to known toxins in a database developed from GenBank using keyword searches for toxins (2018) and to known allergens, gliadin, and glutenin proteins in the "COMprehensive Protein Allergen REsource" (COMPARE) database from the Health and Environmental Sciences Institute (2018).

[3] Relevant sequence similarity was determined by detailed visual inspection, calculated percent identity, E-score ($\leq 1\times10^{-5}$ was deemed significant), and 8 contiguous amino acid identity to a known allergen sequence.

Introduced Protein: Dicamba mono-oxygenase (DMO)

Intended trait	Tolerance to dicamba herbicide
Source organism	*Stenotrophomonas maltophilia*
Protein description	DMO from *S. maltophilia* with an added chloroplast targeting sequence[4]
Intended function	DMO catalyzes the demethylation of dicamba herbicide.

DMO safety assessment

Bayer used an immunoassay to measure the concentration of DMO in MON 87429 corn grain. DMO was detected at a mean level of 2.4 µg/g dry weight.

FDA previously evaluated DMO in consultations BNF 000125, BNF 000135, and BNF 000148. Bayer includes information from those consultations by reference, including information on *in vitro* digestibility, heat stability, and acute oral toxicity. Bayer states that DMO has a documented history of safe consumption; is present at low levels in grain; 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 concludes, based on the weight of the evidence, that dietary exposure to DMO from MON 87429 corn poses no meaningful risk to human health.

In consultation BNF 000125, Bayer investigated whether DMO could catalyze reactions with endogenous substances in the plant and produce unintended reaction products to an extent that would raise food safety questions. The version of DMO assessed in BNF 000125 is identical to DMO from the source organism, except that it has an N-terminal histidine tag. The version of DMO in MON 87429 corn lacks an N-terminal histidine tag, but differs from DMO from the source organism by one or two amino acids at the N-terminus.[4] To ensure these differences do not affect DMO's specificity, Bayer assessed the specificity of the version of DMO from MON 87429 corn on *o*-anisic acid, the endogenous compound identified in BNF 000125 to be structurally most similar to dicamba and therefore considered the most likely to be an unintended substrate of DMO. Using an *in vitro* reaction, liquid chromatography, and mass spectrometry, Bayer found no evidence of DMO activity on *o*-anisic acid. Bayer concludes DMO expressed in MON 87429 corn is unlikely to catalyze reactions with endogenous substances in the plant and produce unintended reaction products to an extent that would raise food safety questions.

[4] Bayer detected a mixture of what it refers to as DMO+0 and DMO+1 in MON 87429 corn. Bayer describes DMO+0 as a form of DMO with an additional leucine at position two relative to the wild-type protein from *S. maltophilia*. Bayer describes DMO+1 as a form of DMO with the same additional leucine as DMO+0 and with an additional cysteine at the N-terminus, from alternative processing of the chloroplast targeting sequence. Bayer does not expect these minor differences in amino acid sequence to impact the protein's safety.

Introduced Protein: Phosphinothricin-N-acetyltransferase (PAT)

Intended trait	Tolerance to glufosinate herbicide
Source organism	*Streptomyces viridochromogenes*
Intended function	PAT catalyzes the acetylation of glufosinate herbicide.

PAT safety assessment

Bayer used an immunoassay to measure the concentration of PAT in MON 87429 corn grain. PAT was detected at a mean level of 0.84 µg/g dry weight.

FDA evaluated PAT in previous consultations, including BNF 000148. The PAT protein expressed in MON 87429 corn is identical to the PAT protein evaluated in BNF 000148. Bayer includes information from BNF 000148 by reference. Bayer states that PAT has a documented history of safe consumption; is present at low levels in grain; lacks similarity to known allergens, toxins, or other biologically active proteins known to have adverse effects on humans; is degraded by digestive enzymes pepsin and pancreatin; loses activity after heat treatment; and is not acutely toxic. Bayer concludes, based on the weight of the evidence, that dietary exposure to PAT from MON 87429 corn poses no meaningful risk to human health.[5]

Bayer states that the PAT protein's mode of action has been evaluated in previous consultations (BNFs 000023, 000028, 000029, 000038, 000046, 000055, 000063, and 000086) and that PAT has high specificity for glufosinate.

Introduced Protein: Modified RdpA (FT_T)

Intended trait	Tolerance to some AOPP ACCase inhibitor herbicides (so called "FOPs" herbicides, such as quizalofop); and tolerance to some synthetic auxin herbicides, including 2,4-D
Source organism	*Sphingobium herbicidovorans*
Protein description	RdpA from *S. herbicidovorans* is described as an α-ketoglutarate-dependent non-heme iron dioxygenase. FT_T is a modified RdpA with a chloroplast targeting sequence and 30 amino acid substitutions to increase enzymatic activity.[6]
Intended function	FT_T catalyzes dioxygenase reactions that degrade certain herbicides.

[5] Although PAT is not used as a plant-incorporated protectant (PIP) inert ingredient in MON 87429 corn, 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.
[6] FDA evaluated the protein AAD-1 in BNF 000120 and NPC 000008. AAD-1, like FT_T, is a version of RdpA from *Sphingobium herbicidovorans*.

Modified RdpA (FT_T)

Bayer used an immunoassay to measure the concentration of FT_T in MON 87429 corn grain. FT_T was detected at a mean level of 47 µg/g dry weight.

Bayer describes the source organism as a ubiquitous bacterium found in soil and water. *Sphingobium* species are not known to cause allergenicity, pathogenicity, or any other adverse health outcomes.

Bayer used bioinformatics analysis to assess FT_T for sequence similarity to known toxins or allergens. Bayer found no structurally relevant similarity with sequences in a toxin protein database containing 28,344 entries from NCBI's GenBank protein database selected by keyword search. Bayer found no structurally relevant similarity with allergen, gliadin, and glutenin protein sequences in the "Comprehensive Protein Allergen Resource" (COMPARE) database (2018) from the Health and Environmental Sciences Institute.

Bayer assessed the stability of FT_T after exposure to heat and to digestive enzymes. FT_T protein remains largely intact and detectable after heat treatment, but becomes inactive after exposure to temperatures of 75°C or greater for 15 minutes. Bayer found that over 99% of FT_T protein is degraded by pepsin and by pancreatin in simulated digestive fluids within 30 seconds, showing that FT_T is rapidly degraded by digestive enzymes.

Bayer conducted an acute oral toxicity study in mice administered FT_T. The study found no evidence of adverse effects at intake levels of up to 2,000 mg/kg body weight, the highest dose tested.

Bayer concludes, based on the weight of the evidence, that dietary exposure to FT_T protein from MON 87429 corn poses no meaningful risk to human health.

Bayer investigated whether FT_T could catalyze reactions with endogenous substances in the plant and produce unintended reaction products to an extent that would raise food safety questions. To do this, Bayer screened the database NAPRALERT,[7] which contains small molecules present in corn, for compounds with structural similarity to the intended herbicide substrates of FT_T. Bayer screened those compounds using computational simulations to identify 38 compounds predicted to be able to bind to FT_T based on FT_T's three-dimensional structure. Of these compounds, 32 were commercially available and were screened *in vitro* as potential substrates for FT_T. Bayer also tested 11 herbicide compounds as positive controls and tested cinnamate, which was previously identified as a marginal substrate for a related enzyme. The assay found that none of the endogenous plant compounds produced a signal significantly exceeding that of negative control reactions lacking a test compound. Data suggest to Bayer that substrates for FT_T require the following structural features: a phenoxy group, a terminal carboxylate, and an available site for oxidation between the phenoxy group and terminal carboxylate. None of the 38 identified compounds possess all three of these characteristics. Bayer concludes that FT_T is unlikely to catalyze reactions with endogenous plant compounds and produce unintended reaction products to an extent that would affect food safety.

[7] https://napralert.org

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

Intended trait	Tolerance to glyphosate herbicide and glyphosate-inducible male sterility
Source organism	*Agrobacterium* sp. strain CP4
Protein description	CP4 EPSPS in MON 87429 corn is identical to wild-type EPSPS from *Agrobacterium* sp. strain CP4, except for the addition of a chloroplast targeting sequence.
Intended function	CP4 EPSPS is functionally equivalent to endogenous plant EPSPS but has reduced affinity for glyphosate. The expression cassette includes an mRNA regulatory element that results in CP4 EPSPS mRNA degradation specifically in tassel tissue. This results in glyphosate susceptibility specifically in tassel tissue, enabling male sterility inducible by appropriately timed glyphosate treatment.

CP4 EPSPS safety assessment

Bayer used an immunoassay to measure the concentration of CP4 EPSPS in MON 87429 corn grain. CP4 EPSPS was detected at a mean level of 0.63 µg/g dry weight.

FDA evaluated CP4 EPSPS in previous consultations, including BNF 000126. Bayer includes information from previous consultations by reference. Bayer also refers to published safety assessments of CP4 EPSPS, which cite studies on acute oral toxicity, digestibility, and heat stability. Bayer states that CP4 EPSPS has a documented history of safe consumption; is present at low levels in grain; 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 concludes, based on the weight of the evidence, that dietary exposure to CP4 EPSPS from MON 87429 corn poses no meaningful risk to human health.[8]

Human Food Nutritional Assessment

The intended traits in MON 87429 corn are not expected to alter levels of key nutrients or anti-nutrients. To assess potential unintended changes in composition relevant to safety or nutrition, Bayer analyzed grain from MON 87429 corn and from a non-genetically engineered (GE) control variety with a similar genetic background, grown in multiple locations in the United States in 2017. Bayer measured proximates (crude protein, crude fat, ash, and carbohydrates by calculation), acid detergent fiber, neutral detergent fiber, total digestible fiber, 18 amino acids, 22 fatty acids, 11 minerals, 7 vitamins, 3 secondary metabolites, and 2 anti-nutrients (phytic acid and raffinose). Bayer found small differences between MON 87429 corn and the non-GE control variety in mean levels of total fat, six fatty acids, copper, iron, magnesium, and alpha-tocopherol. For all of these components, the means for MON 87429 corn were within the range

[8] Although CP4 EPSPS is not used as a PIP inert ingredient in MON 87429 corn, 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 under 40 CFR 174.523.

of variability observed for the non-GE control and within the range of values in the literature and in the International Life Sciences Institute Crop Composition Database 2016 (accessed February 21, 2017).[9] Bayer concludes that grain from MON 87429 corn is compositionally equivalent to the non-GE control variety in levels of key nutrients and anti-nutrients with respect to food safety.

Conclusion

Based on the information provided by Bayer and other information available to CFSAN, we have no further questions at this time about the safety, nutrition, and regulatory compliance of human food from MON 87429 corn. We consider the consultation with Bayer on MON 87429 corn to be complete.

Patrick M. Cournoyer -S

Digitally signed by Patrick M. Cournoyer -S
Date: 2022.06.23 18:13:22 -04'00'

Patrick Cournoyer, PhD

[9] On May 1, 2020, the International Life Sciences Institute Crop Composition Database became known as the Agriculture and Food Systems Institute Crop Composition Database.