Biotechnology Notification File No. 000177
CFSAN Note to the File

Date: September 27, 2022

From: Carrie McMahon, PhD

To: Administrative Record, BNF No. 000177

Subject: Canola with insertion event MON 94100 (MON 94100 canola)

Keywords: Canola, *Brassica napus,* herbicide tolerance, dicamba, demethylase (*dmo*) gene, dicamba mono-oxygenase, DMO, *Stenotrophomonas maltophilia*, Bayer CropScience LP, MON 94100, OECD Unique Identifier MON-941ØØ-2

Summary

Bayer CropScience LP (Bayer)[1] has completed a consultation with the Food and Drug Administration (FDA) on food derived from MON 94100 canola. MON 94100 canola expresses dicamba mono-oxygenase (DMO) for tolerance to dicamba herbicide.[2] This document summarizes Bayer's conclusions and supporting data and information that FDA's Center for Food Safety and Applied Nutrition (CFSAN, 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
- human food from MON 94100 canola is comparable to and as safe as food from other canola varieties

CFSAN evaluated data and information supporting these conclusions and considered whether MON 94100 canola 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 food from MON 94100 canola.

[1] Monsanto Company submitted the notice for BNF No. 000177. 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 residue limits) of pesticidal residues on food.

Subject of the Consultation

Crop	Canola (*Brassica napus*)
Designation	MON 94100
Intended trait	Tolerance to dicamba herbicide
Developer	Bayer CropScience LP
Submission received	January 23, 2020
Amendment received	July 16, 2021
Intended use	General use in human food
Transformation plasmid	PV-BNHT508701
Expression cassette	A demethylase gene (*dmo*) from *Stenotrophomonas maltophilia* encoding dicamba mono-oxygenase (DMO)
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 and sequenced the insertion and flanking genomic sequences using polymerase chain reaction (PCR) and directed sequencing. Sequencing analysis confirmed that the insertion includes a single, intact copy of the T-DNA sequence. Comparison to the untransformed parental variety revealed an 8 base pair deletion of canola genomic DNA at the insertion site.

Absence of vector backbone DNA

Bayer analyzed high throughput sequencing data from MON 94100 canola for the presence of sequences from the vector backbone. Bayer found no sequencing reads that aligned with vector backbone sequences.

Inheritance and stability

Bayer analyzed high throughput sequencing data from five generations of seed-propagated MON 94100 canola. 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 conducted open reading frame analysis of the insertion, as well as the sequences at the junctions between inserted DNA and canola 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 determined that putative polypeptides over eight amino acids in length lacked relevant sequence similarity[3] to known toxins in a database developed from the National Center for Biotechnology Information GenBank using keyword searches (2019) and to known allergens, gliadin, and glutenin proteins in the "COMprehensive Protein Allergen REsource" (COMPARE) database from the Health and Environmental Sciences Institute (2019).

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 enzyme-linked immunosorbent assay (ELISA) to measure the concentration of DMO in MON 94100 canola seed. DMO was detected at a mean level of 0.64 µg/g dry weight. Bayer notes that the only human food currently produced from canola is refined, bleached, and deodorized oil, which contains undetectable amounts of protein.

FDA previously evaluated DMO in consultations BNF 000125, BNF 000135, BNF 000148, and BNF 000173. Bayer states that MON 94100 DMO protein is identical to the DMO protein previously evaluated in BNF 000125. 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 seed; lacks similarity to known allergens, toxins, or other biologically active proteins known to have adverse effects on humans; is not derived from a source organism associated with allergenicity or human or animal pathogenicity; is degraded by the digestive enzymes pepsin and pancreatin; loses activity after heat treatment; and is not acutely toxic. Bayer concludes,

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[3] Relevant sequence similarity was determined by detailed visual inspection, calculated percent identity, E-score ($<1\times10^{-5}$) and, for similarity to known allergens, identity across eight contiguous amino acids.
[4] Bayer describes the sequence of DMO in MON 94100 canola as identical to the wild-type protein from *S. maltophilia*, except for the insertion of an alanine at position 2 and a single amino acid change at position 112 (tryptophan to cysteine). Bayer also describes a second form of DMO in MON 94100 canola with an additional 27 amino acids at the N-terminus derived from the *RbcS* gene due to alternative processing of the chloroplast targeting sequence. *RbcS* is a gene from pea, the source of the chloroplast targeting sequence. Bayer does not expect these differences in amino acid sequence to impact the protein's safety.

based on the weight of the evidence, that dietary exposure to DMO from MON 94100 canola poses no meaningful risk to human health.

Bayer considered 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. Bayer summarized *in vitro* studies conducted to investigate DMO protein activity. In consultation BNF 000125, the results showed that DMO lacked activity on endogenous soybean compounds with structural similarity to dicamba, including *o*-anisic acid, the soybean endogenous compound known to have the greatest structural similarity to dicamba. Bayer also reported no evidence of DMO activity on *o*-anisic acid was observed in studies of the DMO protein in BNF 000173, which is similar but not identical to MON 94100 canola DMO protein. Bayer further conducted a literature search and found no endogenous compounds from canola with structural similarity to dicamba (i.e., phenyl carboxylic acids containing methoxy and chlorine moieties). Bayer concludes DMO is unlikely to catalyze reactions with endogenous substances in canola and produce unintended reaction products that would raise food safety questions.

Human Food Nutritional Assessment

The intended trait in MON 94100 canola is not expected to alter levels of key nutrients or anti-nutrients. To ensure the absence of unintended changes in components relevant to safety or nutrition, Bayer analyzed the composition of seed from MON 94100 canola and from a conventional variety with a similar genetic background (control), grown in multiple locations in the United States and Canada in 2018. Bayer measured proximates (protein, total fat, ash, and carbohydrates by calculation), acid detergent fiber, neutral detergent fiber, amino acids, fatty acids, calcium, phosphorus, vitamin E, vitamin K1, and anti-nutrients (total glucosinolates, total alkyl glucosinolates, total indolyl glucosinolates, phytic acid, sinapine, and tannins).[5] Bayer reported that no statistically significant differences[6] were found for components analyzed in MON 94100 canola and the control, except for sinapine. While the mean level of sinapine for MON 91400 canola was slightly higher than in the control, it was still within the range of values observed for the control and reported in the International Life Sciences Institute Crop Composition Database 2019 (accessed January 15, 2019).[7] Bayer concludes that the difference in sinapine is not biologically meaningful from a food safety perspective and that MON 94100 canola seed is compositionally equivalent to the control variety in levels of the analyzed components.

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[5] Canola varieties of rapeseed (species *B. napus*, *B. rapa*, and *B. juncea*) are low in erucic acid and glucosinolates. See definitions in 7 CFR 810.301 for canola seed and 21 CFR 184.1555(c) for canola oil and Organisation for Economic Co-operation and Development. 2011. Revised consensus document on compositional considerations for new varieties of low erucic acid rapeseed (canola): Key food and feed nutrients, anti-nutrients and toxicants. ENV/JM/MONO (2011)55. OECD, Paris, France.

[6] T-test analyses were used to determine statistical significance at the level of $P \leq 0.05$ for differences between MON 94100 canola and the control.

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

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 94100 canola. We consider the consultation with Bayer on MON 94100 canola to be complete.

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