



Biotechnology Notification File No. 000186

CFSAN Note to the File

Date: June 8, 2023

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To: Administrative Record, BNF No. 000186

Subject: Corn with transformation event MON 95275 (MON 95275 corn)

Keywords: Corn, maize, *Zea mays*, resistance to targeted coleopteran insect pests, western and northern corn rootworm, *mpp75Aa1.1* gene, Mpp75Aa1.1 protein (formerly Cry75Aa1.1), *Brevibacillus laterosporus*, *vpb4Da2* gene, Vpb4Da2 protein (formerly Vip4Ba1/Vip4Da2), *Bacillus thuringiensis*, double-stranded RNA transcript, *DvSnf7.1*, *Diabrotica virgifera virgifera*, Bayer CropScience LP, MON 95275, OECD Unique Identifier MON-95275-7

Summary

Bayer CropScience LP (Bayer) has completed a consultation with the Food and Drug Administration (FDA) on food derived from MON 95275 maize (corn) genetically engineered to express: the *Brevibacillus laterosporus* Mpp75Aa1.1 protein, the *Bacillus thuringiensis* Vpb4Da2 protein,¹ and the *DvSnf7.1* double stranded RNA (dsRNA) derived from the western corn rootworm. These modifications are intended to confer resistance to targeted coleopteran insect pests, including western corn rootworm and northern corn rootworm. 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 of MON 95275 corn. 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 95275 corn is comparable to and as safe as human food from other corn varieties.

¹ The Mpp75Aa1.1 and Vpb4Da2 proteins expressed in MON 95275 corn were previously known as Cry75Aa1.1 and Vip4Ba1/Vip4Da2, respectively. The reclassification of these two proteins were based on structural homology. Crickmore, N. et al., 2021. A structure-based nomenclature for *Bacillus thuringiensis* and other bacteria-derived pesticidal proteins. *Journal of Invertebrate pathology*, 186: 107438.

CFSAN evaluated data and information supporting these conclusions and considered whether MON 95275 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 95275 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. 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.” In MON 95275 corn, the Mpp75Aa1.1 and Vpb4Da2 proteins, and the *DvSnf7.1* dsRNA are PIPs. Bayer explained that it will seek an exemption from the requirement of a tolerance for the PIP proteins Mpp75Aa1.1 and Vpb4Da2 in or on food for humans and animals that is derived from Mon 95275 corn. Bayer also stated that the *DvSnf7.1* dsRNA falls under the established exemption from the requirement of a tolerance for residues of nucleic acids that are part of a PIP (40 CFR 174.507, redesignated § 174.475, effective April 25, 2007). The safety of Mpp75Aa1.1 and Vpb4Da2 proteins and the *DvSnf7.1* dsRNA in MON 95275 corn falls under EPA’s purview and therefore is not addressed in this document.

Subject of the Consultation

Crop	Corn
Designation	MON 95275
Intended trait	Insect resistance
Developer	Bayer CropScience LP
Submission received	November 4, 2021
Amendments received	March 17 and 30, 2022; December 2, 2022
Intended use	For general use in human food
Transformation plasmid	plasmid PV-ZMIR525664
Expression cassette 1	The <i>mpp75Aa1.1</i> expression cassette contains the coding sequence of Mpp75Aa1.1 protein from <i>Brevibacillus laterosporus</i> .
Expression cassette 2	The <i>vpb4Da2</i> expression cassette contains the coding sequence of Vpb4Da2 protein from <i>Bacillus thuringiensis</i> .
Expression cassette 3	The <i>DvSnf7.1</i> suppression cassette contains the inverted repeat nucleotide sequence of the <i>Snf7</i> gene from <i>Diabrotica virgifera</i> encoding the SNF7 subunit of the ESCRT-III complex. The cassette produces a

	240-base pair dsRNA transcript that induces an RNAi mechanism in the pest.
Expression cassette 4	The selectable <i>cp4 epsps</i> marker cassette contains the coding sequence of CP4 EPSPS protein from <i>Bacillus thuringiensis</i> . This cassette is flanked by two excision targeting sequences called <i>lox</i> sites for marker removal.
Method for conferring genetic change	<i>Agrobacterium</i> -mediated transformation

Inheritance and stability

Bayer first introduced the T-DNA containing all four cassettes into immature embryos of the recipient corn line using *Agrobacterium*-mediated transformation and then crossed MON 95275 corn (R2 progeny)² with a corn variety expressing the Cre recombinase³ to remove the selectable *cp4 epsps* marker cassette. The Cre recombinase in the resulting F1 progeny initiated the excision of the selectable marker cassette containing *cp4 epsps* gene flanked by the *loxP* sites. Conventional breeding was subsequently used to remove the *cre* gene and its associated elements. Bayer confirmed the absence of *cp4 epsps* cassette, *cre* gene, and its associated genetic elements in F2 progeny by directed sequencing. Bayer performed a combination of high-throughput sequencing, junction sequence analysis, and bioinformatics analysis to confirm the integrity and determine the copy number of the inserted T-DNA in five generations (F4, F4F1, F5, F5F1, and F6) of MON 95275 corn. Bayer identified the same two junction sequences in each generation, confirming the stable inheritance of the single insertion. By using PCR genotyping and chi-square analysis of segregating progeny, Bayer concluded that MON 95275 corn contains one copy of the T-DNA (without the *cp4 epsps* marker cassette) that resides at a single locus, is stably inherited over multiple generations, and segregates according to Mendelian principles.

Human Food Nutritional Assessment

The intended trait in MON 95275 corn is not expected to alter levels of key nutrients or anti-nutrients. To assess potential unintended changes relevant to safety or nutrition of human food, Bayer compared the composition of grain from MON 95275 corn (hybrid) to grain from a conventional control hybrid variety⁴ grown in five locations in the United States in 2019. Bayer analyzed the grain for levels of nutrients including proximates (protein, fat, total dietary fiber, ash, and carbohydrates (by calculation)) as well as amino acids, fatty acids, acid detergent fiber, neutral detergent fiber, minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc), and vitamins (vitamin A (as β -carotene) and vitamins B1, B2, B3,

² The transformed RO plant containing the MON 95275 event was self-pollinated to generate R1 seed. The homozygous positive R1 plant was self-pollinated to generate R2 seed. The R2 plant was crossed with the Cre recombinase expressing line to give F1 seed. F1 progeny was self-pollinated to give F2 seed. F2 plants were used to screen for the absence of *cre* gene.

³ Hare, P.D. and N.-H. Chua. 2002. Excision of selectable marker genes from transgenic plants. *Nature Biotechnology* 20:575-580.

⁴ A conventional control hybrid corn variety that has a similar genetic background to that of MON 95275 corn hybrid variety was used for compositional analysis.

B6, B9, and E). Bayer also analyzed the collected samples for levels of anti-nutrients (phytic acid and raffinose) and secondary metabolites (ferulic acid and *p*-coumaric acid).⁵

Bayer observed statistical differences in the mean levels ($p < 0.05$) between MON 95275 corn and the control grain for palmitic acid, stearic acid, oleic acid, linoleic acid, arachidic acid, calcium, and vitamin B6. Bayer states that the mean levels for these seven components were within the ranges observed for the control and levels reported in the International Life Sciences Institute Crop Composition Database version 7.0.⁶ The mean levels for the seven components were also within or similar to levels in published literature cited by Bayer. Bayer concludes that these differences are within the expected natural variability of corn composition and that the minor differences are not relevant to human nutrition. Bayer further concludes that MON 95275 corn is as safe as other corn varieties.

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 95275 corn. We consider the consultation with Bayer on MON 95275 corn to be complete.

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⁵ Bayer excluded 15 components from the statistical analysis because of their low-level presence (below the LOQ). These components were not discussed in this document.

⁶ On May 1, 2020, the International Life Sciences Institute Crop Composition Database became known as the Agriculture and Food Systems Institute Crop Composition Database.