U.S. FOOD & DRUG ADMINISTRATION

Biotechnology Notification File No. 000168
CFSAN Note to the File

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To: Administrative Record, BNF No. 000168

Subject: Glyphosate and glufosinate-ammonium tolerant DBN9858 corn

Keywords: Corn, Maize, *Zea mays*, herbicide tolerance, glyphosate, glufosinate-ammonium, 5-enolpyruvylshikimate-3-phosphate synthase, EPSPS, phosphinothricin acetyltransferase, PAT, *Agrobacterium* sp. strain CP4, *Streptomyces viridochromogenes*, Beijing DaBeiNong Biotechnology Co. Ltd., DBNBC, OECD unique identifier DBN-Ø9858-5

Summary

Beijing DaBeiNong Biotechnology Co. Ltd. (DBNBC) has completed a consultation with the Food and Drug Administration (FDA) on food derived from herbicide tolerant DBN9858 corn that expresses a modified 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) derived from *Agrobacterium* sp. strain CP4 and the phosphinothricin acetyltransferase (PAT) derived from *Streptomyces viridochromogenes*. This document summarizes DBNBC'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 in a separate document.

DBNBC concludes:

- it has not introduced into food a new protein or other substance that would require premarket approval as a food additive
- human food from herbicide tolerant corn is comparable to and as safe as human food from other corn
- the majority of the herbicide tolerant DBN9858 corn will be used in China for domestic use and a small amount may be present in Chinese-produced food products that may be exported

We evaluated data and information supporting these conclusions and considered whether DBN9858 corn raises other regulatory issues involving human food 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 DBN9858 corn.

Subject of the Consultation

Crop:	Corn
Designation:	DBN9858
Intended trait:	Tolerance to glyphosate herbicides
Intended trait:	Tolerance to glufosinate-ammonium herbicides
Developer:	Beijing DaBeiNong Biotechnology Co. Ltd. (DBNBC)
Original submission received:	July 9, 2018
Amendments received:	March 19, 2019, March 29, 2019, July 1, 2019, and December 17, 2020
Intended use:	Corn and its by-products
Transformation vector:	pDBN0010
Expression cassette 1:	*epsps* cassette consisting of the *epsps* coding sequence derived from *Agrobacterium* sp. strain CP4 fused to the *epsps chloroplast transit peptide* coding sequence derived from *Arabidopsis thaliana*
Expression cassette 2:	*pat* cassette consisting of the *pat* coding sequence from *S. viridochromogenes*
Transformation method	*Agrobacterium*-mediated transformation

Molecular Characterization

Confirmation of intended genetic change

DBNBC used PCR analysis and DNA sequencing to confirm the integrity and to determine the copy number of the inserted DNA. DBNBC also showed that DBN9858 corn contained the intact transfer T-DNA from pDBN0010 and a 46 bp deletion from the corn genome at the integration site. This analysis also showed that only a single copy of T-DNA was inserted and that no plasmid backbone sequences were inserted. The latter results were confirmed by Southern blot analysis, which showed the presence of the donor genes and absence of the vector backbone elements.

Open reading frame analysis

Analysis of the DNA sequences spanning the 5' and 3' junctions between the corn genomic sequence and the DBN9858 insert did not identify any nucleotide sequences contained between a start codon (ATG) and a stop codon (TAG, TAA, or TGA) whose translated hypothetical open reading frame (ORF) was ≥30 amino acids.

Stability over multiple generations

DBNBC performed Southern blot analysis across five generations of seed-propagated DBN9858 corn and found consistent hybridization patterns, confirming the stability of the intended genetic change. The desired phenotype of DBN9858 corn was also stably maintained across five generations. Further, donor gene-specific PCR confirmed that *epsps* and *pat* genes were stably integrated into the DBN9858 corn genome and were inherited across five generations in a Mendelian fashion.

Introduced Protein: EPSPS

Intended trait	Tolerance to glyphosate herbicides
Source organism	*Agrobacterium* sp. strain CP4
Protein description	5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), fused to the chloroplast transit peptide of EPSPS derived from *Arabidopsis thaliana*
Intended function	EPSPS catalyzes the transfer of the enolpyruvyl moiety of phosphoenolpyruvate to the 5-hydroxy position of shikimate-3-phosphate. CP4 EPSPS has reduced affinity to glyphosate (compared to endogenous EPSPS)

EPSPS Safety Assessment

Apart from the reduced affinity for glyphosate, the EPSPS expressed in DBN9858 corn is functionally and structurally equivalent to endogenous EPSPS and is also similar to the other diverse and ubiquitous EPSPS family members found in other plants and bacteria. There is a long history of safe consumption of plant- and microbe-based foods and by extension consumption of EPSPS.

DBNBC characterized EPSPS by determining its molecular weight, immunoreactivity, and amino acid sequence. The potential for toxicity and allergenicity of EPSPS expressed in DBN9858 corn was assessed by comparing it to known allergens and toxins.[1] DBNBC also determined the thermostability of EPSPS, its susceptibility to rapid enzymatic degradation in simulated gastric fluid (SGF), and its glycosylation status. A mouse acute oral toxicity study using high doses of microbially-expressed EPSPS protein was utilized to test for acute toxicity.

DBNBC analysis of EPSPS revealed that the protein does not share any significant sequence similarity or relatedness to known allergens or toxins and the corn-expressed protein is not glycosylated. The EPSPS protein was shown to be highly labile in SGF and was rapidly hydrolyzed within a few minutes of incubation. No signs of adverse effects were observed in

[1] For both EPSPS and PAT, the allergenic potential of the proteins was assessed by comparison with known and putative allergens using the Food Allergy Research and Resource Program (FARRP) AllergenOnline.org database. The Basic Local Alignment Search Tool for Proteins (BLASTP) from the National Center for Biotechnology Information (NCBI) was used to search the NCBI protein database for sequence similarity between the proteins and known toxins.

mice that were administered EPSPS protein. Although not a plant-incorporated protectant in this application, the safety of EPSPS protein is also supported by a U.S. Environmental Protection Agency (EPA) exemption from tolerance requirements in all food commodities under 40 CFR 174.523.

Introduced Protein: Phosphinothricin-N-acetyltransferase (PAT)

Intended trait	Tolerance to glufosinate herbicides
Source organism	*S. viridochromogenes*
Intended function	PAT catalyzes the acetylation of phosphinothricin, the active component in glufosinate herbicides

PAT Safety Assessment

The *pat* gene was obtained from *S. viridochromogenes,* which is a non-pathogenic soil-borne bacterium. DBNBC stated that the safety of PAT has been affirmed in a peer-reviewed study[2] and that PAT is used for herbicide tolerance in commercially available crops such as corn, canola, and soybean. The safety of PAT is also supported by a U.S. EPA exemption from tolerance requirements in all food commodities (40 CFR 174.522). Humans are also exposed to similar acetyltransferases from various sources either in food or the endogenous microbiome.

DBNBC characterized PAT by determining its molecular weight, immunoreactivity, and amino acid sequence. The potential for toxicity and allergenicity of PAT expressed in DBN9858 corn was assessed by comparing it to known allergens and toxins.[1] DBNBC also determined the thermostability of PAT, its susceptibility to rapid enzymatic degradation in SGF, and its glycosylation status. A mouse acute oral toxicity study using high doses of microbially-expressed PAT was utilized to test for acute toxicity.

DBNBC analysis of PAT revealed that the protein does not share any significant sequence similarity to known allergens or toxins and the corn-expressed protein is not glycosylated. The PAT protein was rapidly hydrolyzed within a few minutes of incubation in SGF. No signs of adverse effects were observed in mice that were administered PAT protein.

Human Food Nutritional Assessment

The intended traits in DBN9858 corn are not expected to alter levels of key nutrients, anti-nutrients, or toxicants. To ensure the absence of unintended changes in components relevant to safety or nutrition of human food from DBN9858 corn, DBNBC analyzed corn grain from DBN9858 and the non-genetically engineered parental variety (control line 178) for key components. DBNBC measured levels of proximates (moisture, ash, carbohydrates, fat, protein, coarse fiber), anti-nutrients (phytic acid and trypsin inhibitor), amino acids, minerals, and

[2] Hérouet C, et al., (2005). Safety evaluation of the phosphinothricin acetyltransferase proteins encoded by the *pat* and *bar* sequences that confer tolerance to glufosinate-ammonium herbicide in transgenic plants. Regulatory Toxicology and Pharmacology 41:134-149.

vitamins. DBNBC observed that the levels of the components in DBN9858 and control line 178 were similar and were within ranges of variation reported in the literature (ILSI crop database[3], and OECD[4]). DBNBC concludes that DBN9858 corn is compositionally and nutritionally comparable to conventional corn varieties.

Conclusion

Based on the information provided by DBNBC and other information available to FDA, we have no further questions at this time about the safety, nutrition, and regulatory compliance of human food from DBN9858 corn. We consider the consultation with DBNBC on DBN9858 corn to be complete.

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[3] International Life Sciences Institute Crop Composition Database (2018). On May 1, 2020, the International Life Sciences Institute Crop Composition Database became known as the Agriculture and Food Systems Institute Crop Composition Database.
[4] OECD. 2002. Consensus Document on Compositional Considerations for New Varieties of Maize (*Zea mays*): Key Food and Feed Nutrients, Anti-nutrients and Secondary Plant Metabolites. ENV/JM/MONO(2002)25. Organisation for Economic Co-operation and Development, Paris, France.