



Biotechnology Notification File No. 000170 CVM Note to the File

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To: Administrative Record, BNF No. 000170

Subject: Event IND-ØØ412-7 Wheat

Keywords: Wheat, *Triticum aestivum*, modified *HaHB4* transcription factor, *Helianthus annuus*, sunflower, tolerance to environmental stresses, phosphinothricin acetyltransferase, PAT, *bar*, *Streptomyces hygrosopicus*, herbicide tolerance, glufosinate ammonium, OECD Unique Identifier: IND-ØØ412-7, Bioceres Inc., New Protein Consultation (NPC) 000016

Purpose

This document summarizes the Food and Drug Administration (FDA) Center for Veterinary Medicine's (CVM, we) evaluation of biotechnology notification file (BNF) number 000170. Bioceres Inc. (Bioceres) submitted a safety and nutritional assessment for a genetically engineered (GE) wheat, transformation event IND-ØØ412-7 (IND-ØØ412-7 wheat), and additional information afterwards.

Bioceres states that IND-ØØ412-7 wheat is intended solely for cultivation in Argentina or South America. There is no plan to grow IND-ØØ412-7 wheat in the United States. In addition, Bioceres anticipates that the presence of IND-ØØ412-7 wheat in food in the United States would be limited to processed products.

CVM evaluated the information in Bioceres' submissions to ensure that regulatory and safety issues regarding the intermittent low levels of IND-ØØ412-7 wheat in animal food in the United States have been resolved prior to commercial distribution. FDA's Center for Food Safety and Applied Nutrition summarizes its evaluation of IND-ØØ412-7 wheat in human food in a separate document.

In CVM's evaluation, we considered information provided by Bioceres as well as publicly available information and information in the agency's files.

Intended Effects

The intended effects of the modifications in IND-ØØ412-7 wheat are to increase tolerance to environmental stresses that reduce crop yield and confer tolerance to the herbicide glufosinate ammonium. To confer tolerance to environmental stresses,

Bioceres introduced a modified *HaHB4* gene from common sunflower (*Helianthus annuus*), which encodes a HAHB4 transcription factor¹. Bioceres states that HAHB4 is actively involved in the response of plants to abiotic stress, which enables yield improvement compared to controls under environmental stress. In addition, Bioceres introduced the *Streptomyces hygrosopicus bar* gene, which encodes for the phosphinothricin acetyltransferase (PAT/*bar*) protein that confers tolerance to the herbicide glufosinate ammonium.

Regulatory Considerations

The purpose of this evaluation is to determine whether the intermittent low levels of the new plant variety in animal food raises safety or regulatory issues under the Federal Food, Drug, and Cosmetic Act (FD&C Act).

The Environmental Protection Agency (EPA) regulates herbicides under the FD&C Act and the Federal Insecticide, Fungicide, and Rodenticide Act. Under EPA regulations, the herbicide residues in IND-ØØ412-7 wheat are considered pesticidal residues.

Animal Food Use

In its submissions, Bioceres states that it does not intend to grow IND-ØØ412-7 wheat in the United States. As a consequence, Bioceres states that it has not requested non-regulated status for IND-ØØ412-7 wheat from the United States Department of Agriculture.

Currently, wheat intended for use in animal food is not typically imported into the United States from Argentina or South America. Under these conditions, IND-ØØ412-7 wheat from Argentina or South America may only be present in animal food in intermittent low levels. The primary food safety considerations under these conditions are the safety of any new substances introduced into the food.²

Wheat (*Triticum aestivum*), a hexaploid species, has been cultivated for use in food for several millennia and is the most widely grown crop in the world with the United States being a major exporting country. The majority of wheat grain is used for human food, mainly for the production of flour which is used in baked goods or other flour-based foods. The use of wheat as animal food is minor when compared to its use in human food. Wheat grain and its by-products from the production of human food are used as animal food. Wheat forage is also used as an animal food and can be used for grazing cattle. Wheat straw is also commonly used as bedding for animals and can be consumed.

¹ HAHB4 is the subject of New Protein Consultation No. (NPC 000016). FDA responded to NPC 000016 on August 7, 2015.

² Guidance for Industry: Recommendations for the Early Food Safety Evaluation of New Non-Pesticidal Proteins Produced by New Plant Varieties Intended for Food Use, available at <https://www.fda.gov/regulatory-information/search-fda-guidance-documents/guidance-industry-recommendations-early-food-safety-evaluation-new-non-pesticidal-proteins-produced>.

Protein Safety

IND-ØØ412-7 wheat was genetically engineered to express a HAHB4 protein derived from sunflower (*Helianthus annuus*). HAHB4 is a transcription factor belonging to the homeodomain-leucine zipper I (HD-Zip I) family, unique to plants, and characterized as important in the response of plants to abiotic stress.³ Bioceres states that the HAHB4 protein in IND-ØØ412-7 wheat has a four amino acid deletion and three amino acid substitutions⁴ compared to the original sunflower HAHB4 sequence, but is identical to the HAHB4 protein that was expressed in new plant variety (BNF No. 000155) that has previously been evaluated for safety by FDA.

IND-ØØ412-7 wheat was also genetically engineered to express the PAT/*bar* protein, which confers tolerance to the herbicide glufosinate ammonium. Bioceres states the PAT/*bar* protein in IND-ØØ412-7 wheat is encoded by the *bar* gene isolated from *Streptomyces hygroscopicus* and is identical to other PAT/*bar* proteins expressed in several plant varieties that have completed FDA's consultation program.

To assess the safety of the introduced proteins⁵, Bioceres conducted bioinformatics analyses of the HAHB4 amino acid sequence and concludes that HAHB4 protein in IND-ØØ412-7 wheat has no significant amino acid similarity to known toxins which is consistent with the conclusions in BNF No. 000155 and NPC 000016. Bioceres reports that HAHB4 protein levels are present at extremely low concentrations in IND-ØØ412-7 wheat leaf and grain⁶. Bioceres also measured PAT/*bar* protein levels in IND-ØØ412-7 wheat and states these levels fell within the range of values observed in the literature. Bioceres concludes that the safety of PAT/*bar* protein has been previously established based on the scientific literature⁷, consultations completed by FDA, human and animal food approvals by other governments for crops containing either the *pat* or *bar* genes.

Based on the information provided in previous BNFs, the information discussed above, and in its submissions, Bioceres concludes that the HAHB4 and PAT/*bar* proteins in IND-ØØ412-7 wheat do not raise safety concerns.

³ Dezar, C. A., Gago, G. M., Gonzalez, D. H., Chan, R. L. 2005. Hahb-4, a sunflower homeobox-leucine zipper gene is a developmental regulator and confers drought tolerance to *Arabidopsis thaliana* plants. *Transgenic Res.* 14: 429-440.

⁴ Bioceres concludes that the effects of these changes are not expected to produce significant differences in the general properties of the HAHB4 protein.

⁵ Bioceres performed digestibility in simulated gastric fluid, thermal stability, and glycosylation analyses for its allergenicity assessment. Bioceres demonstrated the equivalence of the plant-expressed and *E. coli*-expressed proteins using mass spectrometry and N-terminal sequence analyses.

⁶ Bioceres developed a specific LC-MS/MS method to detect HAHB4 expression levels. In non-stress conditions, HAHB4 protein levels in IND-ØØ412-7 wheat are lower than the limit of detection in leaf tissue and grain. Under abiotic stress, HAHB4 expression levels range from 0.011 to 0.015 ng/g FW leaf tissue in IND-ØØ412-7 wheat.

⁷ Hérouet, C., D.J. Esdaile, B.A. Mallyon, E. Debruyne, A. Schulz, T. Currier, K. Hendrickx, R.J. van der Klis and D. Rouan. 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. *Regul. Toxicol. Pharmacol.* 41: 134-149.

Conclusion

CVM has evaluated potential safety and regulatory issues associated with intermittent low levels of IND-ØØ412-7 wheat in animal food; CVM has not evaluated IND-ØØ412-7 wheat for general use in animal food. It is Bioceres's responsibility to ensure that any expressed proteins present in IND-ØØ412-7 wheat are safe and legal when present in animal food in the United States. Based on the information Bioceres has provided to FDA, CVM has no questions concerning the safety of intermittent low levels of IND-ØØ412-7 wheat in animal food. CVM recommends that should Bioceres or distributors of IND-ØØ412-7 wheat intend to market the wheat or its by-products as animal food in the United States, they should contact CVM's Division of Animal Food Ingredients.

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