Biotechnology Notification File No. 000156
Note to the File

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From: Patrick Cournoyer

To: Administrative Record, BNF No. 000156

Subject: Huahui No.1 insect resistant rice

Keywords:
Rice, *Oryza sativa*, Huahui No. 1, Bt Shanyou63, T51/TT51, T51-1/TT51-1, Cry1Ab/Cry1Ac, *Bacillus thuringiensis* subsp. Kurstaki, Insect resistance, Huazhong Agricultural University

Purpose

This document summarizes the Food and Drug Administration's (FDA, our) evaluation of biotechnology notification file (BNF) No. 000156. Huazhong Agricultural University (the developer) submitted a safety and nutritional assessment of genetically engineered (GE) Huahui No.1 rice grain, which we received on June 13, 2016.[1,2] The developer provided additional information on January 8, 2017. We evaluated the information in the developer's submissions to ensure that regulatory and safety issues regarding human and animal food from Huahui No.1 rice grain[3] have been resolved prior to commercial distribution.

In our evaluation, we considered all information provided by the developer as well as publicly available information and information in the agency's files. Here, we discuss the outcome of the consultation, but do not intend to restate the information provided in the final consultation in its entirety.

Intended Effects

The intended effect of the modification in Huahui No.1 rice is protection against lepidopteran insect pests of rice in China. To accomplish this objective, the developer introduced a gene encoding the chimeric protein Cry1Ab/Cry1Ac, which is a fusion of Cry1Ab from *Bacillus thuringiensis* subsp. *kurstaki* strain HD-1 and Cry1Ac from *B. thuringiensis* subsp. *kurstaki* strain HD-73. Cry1Ab/Cry1Ac is toxic to lepidopteran insect pests of rice.

[1] Rice varieties containing the genetic insertion in Huahui No.1 rice have also been referred to as Bt63, Bt Shanyou 63, BtSY63, T51, TT51, T51-1, and TT51-1.
[2] In its submission, Huazhong Agricultural University provided information on brown rice from Huahui No. 1 rice, but not straw or hulls.
[3] The developer informed FDA that Huahui No. 1 rice would be cultivated in China and that a very small proportion of rice is exported to the United States as processed food products.

Regulatory Considerations

The purpose of this evaluation is to assess whether the developer has raised safety or regulatory issues under the Federal Food, Drug and Cosmetic Act (FD&C Act).

The United States Environmental Protection Agency (EPA) defines a plant-incorporated protectant (PIP) 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" (40 CFR 174.3). EPA regulates PIPs under the FD&C Act and the Federal Insecticide, Fungicide, and Rodenticide Act. Under EPA regulations, the *cry1Ab/cry1Ac* gene in Huahui No.1 rice and resulting expression product are considered pesticidal substances. The developer provided a copy of a letter from EPA stating that an existing tolerance exemption applies to the Cry1Ab/Cry1Ac protein in Huahui No. 1 rice.

Inheritance and Stability

Huazhong Agricultural University confirmed the genomic stability of the inserted DNA over multiple generations.[4] The developer used Southern blot analysis to detect the inserted DNA in plants derived from the initially transformed tissue, in plants eight generations after transformation, and in a hybrid derived from Huahui No.1. Based on observing consistent Southern blots in each generation, the developer concludes that the inserted DNA in Huahui No.1 rice is stably inherited.[5]

Huazhong Agricultural University performed bioinformatics analyses using the sequence of the DNA insert and the flanking genomic sequences to identify putative new open reading frames (ORFs) and to assess the potential of putative peptides encoded by these putative ORFs to be an allergen or a toxin. The developer reports that none of the ORFs would encode peptides with similarity to known allergens or toxins.

Human and Animal Food Use

Huazhong Agricultural University refers to the OECD Consensus Document on Compositional Considerations for New Varieties of Rice for information about the uses of rice grain in human and animal food.[6] According to this document, humans mainly consume rice in the form of cooked grain, which may be brown (hull has been removed), milled (bran has been removed), or polished. Rice may also be used to produce ingredients in prepared foods, which are widely found and consumed in Asia. Animals can be fed paddy rice, silage from whole rice plants, and by-products, including straw, hull, and bran, which are obtained during the processing of the grain.

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[4] Huazhong Agricultural University transformed Huahui No.1 rice by particle bombardment with the plasmid containing the *cry1Ab/cry1Ac* sequence as well as a second plasmid containing the selectable marker gene Hygromycin B phosphotransferase (*hph*) and the rice chitinase gene *RC7*. The developer eliminated *hph* from the T1 generation by self-pollination and genetic segregation, which was confirmed by PCR analysis. The developer did not screen for the absence of the *RC7* gene, but expects it would be absent because it would likely be genetically linked to *hph*.

[5] FDA also referenced a scientific publication, which describes bioinformatics analyses of whole genome sequence data obtained for Huahui No. 1 rice and the results of PCR amplification of the insertion sites followed by Sanger sequencing. Yang, L., C. Wang, A. Holst-Jensen, D. Morisset, Y. Lin, and D. Zhang. 2013. Characterization of GM events by insert knowledge adapted re-sequencing approaches. Sci. Rep. 3:2839.

[6] OECD. 2016. Revised Consensus Document on Compositional Considerations for New Varieties of Rice (*Oryza sativa*): Key Food and Feed Nutrients, Anti-nutrients and Other Constituents. ENV/JM/MONO(2016)38. Organisation for Economic Co-operation and Development, Paris, France.

Composition

Scope of Analysis

Huazhong Agricultural University compared the composition of Huahui No.1 rice to the non-GE, parental rice variety Minghui 63 (control). The developer also compared levels of components measured in Huahui No. 1 rice to values from other rice varieties reported in the scientific literature and in publicly available databases.

Study Design - Compositional Analyses

Huazhong Agricultural University grew Huahui No.1 rice and the control at three locations in rice-growing regions of China in 2008. At each location, the control and Huahui 1 rice were grown in adjacent plots and were subject to normal field management practices. The developer collected five grain samples from the control and from Huahui No. 1 rice at each location. Samples of each variety were pooled and dehulled to obtain unpolished, brown rice for analysis. The developer presents values of two replicates per pooled sample.

The developer measured levels in the grain of proximates (moisture, crude protein, crude fat, ash, and carbohydrates by calculation), starch (total starch, amylose, and amylopectin), vitamins (A, B1, B2, B5, E, and folate), minerals (magnesium, potassium, calcium, zinc, copper, and phosphorus), anti-nutrients (trypsin inhibitor and phytic acid), heavy metals (arsenic, mercury, lead, cadmium, and selenium), and 17 amino acids. [7] Moisture values were used to express the data on a dry matter basis.

The developer presented values for each analyzed sample as well as mean values for each variety at each site. A paired t-test was used to statistically analyze differences in components in grain obtained from Huahui No. 1 and the control. Vitamin A and folate were excluded from statistical analyses because their levels in the rice grain samples fell below the limit of quantitation.

Results of analyses

The developer found no statistically significant differences between Huahui No.1 rice and the control in the across-site analyses. The developer concludes that Huahui No.1 rice grain is compositionally equivalent to the non-GE comparator.[8]

Conclusion

FDA evaluated Huazhong Agricultural University's submission to determine whether Huahui No.1 rice grain raises any safety or regulatory issues with respect to its use in human and animal food. Based on the information provided by the company and other information available to the agency, FDA did not identify any safety or regulatory issues with food derived from Huahui No. 1 rice grain under the FD&C Act that would require further evaluation at this time.

Huazhong Agricultural University has concluded that grain derived from its insect-resistant rice variety, Huahui No.1 rice, and the human and animal foods derived from it are as safe as grain derived from

[7] Huazhong Agricultural University did not measure levels of fiber, vitamin B3, vitamin B6, and tryptophan. We note that Huahui No. 1 rice was engineered to express the protein Cry1Ab/Ac, which is not intended to alter rice grain composition. The overall compositional analysis performed by the developer supports a lack of meaningful compositional changes in Huahui No. 1 rice. Levels of 17 other amino acids did not differ from the control, making differences in levels of tryptophan unlikely. Furthermore, rice products imported into the US from China for use in human diet are not significant sources of fiber, vitamin B3, vitamin B6, and tryptophan. Rice products for human consumption imported from China are typically made from polished, white rice, which lacks significant levels of fiber.

[8] In addition to the compositional data presented by the developer for Huahui No. 1 rice and the control, FDA also reviewed literature values for composition of conventional rice varieties. FDA did not observe any meaningful differences in composition that would affect human or animal safety or nutrition.

conventional rice varieties and are not materially different in composition or any other relevant parameter from other rice varieties now grown, marketed, and consumed. At this time, based on Huazhong Agricultural University's data and information, the agency considers developer's consultation on Huahui No.1 rice grain to be complete.

Patrick M. Cournoyer -S

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Patrick Cournoyer, Ph.D.