



Gary L. Yingling
Morgan, Lewis & Bockius LLP
1111 Pennsylvania Avenue, NW
Washington, DC 20004

Re: GRAS Notice No. GRN 000737

Dear Mr. Yingling:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 000737. We received the notice that you submitted on behalf of Impossible Foods Inc. on October 3, 2017,¹ and filed it on October 26, 2017. Impossible Foods submitted amendments to the notice on November 29 and December 5, 2017; and February 27, March 6, June 29, and July 10, 2018. In the amendments, the notifier informs FDA of the publication status of two scientific articles and clarifies the intended conditions of use of soy leghemoglobin preparation.

The subject of the notice is soy leghemoglobin preparation from a strain of *Pichia pastoris* (soy leghemoglobin preparation) for use at a level up to 0.8% soybean leghemoglobin protein to optimize flavor in ground beef analogue products intended to be cooked. The notice informs us of Impossible Foods' view that this use of soy leghemoglobin preparation is GRAS through scientific procedures.

Our use of the term, "soy leghemoglobin preparation," in this letter is not our recommendation of that term as an appropriate common or usual name for declaring the substance in accordance with FDA's labeling requirements. Under 21 CFR 101.4, each ingredient must be declared by its common or usual name. In addition, 21 CFR 102.5 outlines general principles to use when establishing common or usual names for nonstandardized foods. Issues associated with labeling and the common or usual name of a food ingredient are under the purview of the Office of Nutrition and Food Labeling (ONFL) in the Center for Food Safety and Applied Nutrition. The Office of Food Additive Safety (OFAS) did not consult with ONFL regarding the appropriate common or usual name for "soy leghemoglobin preparation."

Impossible Foods describes soy leghemoglobin preparation as a mixture containing soy leghemoglobin protein, *P. pastoris* proteins, sodium chloride, and sodium ascorbate. The soy leghemoglobin preparation is red/brown. The preparation is produced using

¹ Impossible Foods provided an update to its notice on October 18, 2017. The update includes information about the intended use of soy leghemoglobin preparation.

P. pastoris production strain MXY0291, which was constructed from the commercially available *P. pastoris* Bg11 strain. Impossible Foods describes *P. pastoris* as a non-pathogenic, non-toxic, and well-characterized yeast with a history of safe use in the food industry.

Impossible Foods describes the construction of the production strain through transformation of the recipient Bg11 strain using (1) multiple copies of a codon-optimized gene encoding the leghemoglobin LGB2 apoprotein from soybean (*Glycine max*), (2) additional copies of eight *P. pastoris* genes encoding enzymes catalyzing heme B biosynthesis, and (3) transcriptional regulatory elements to improve protein production. The expression of these genes results in overexpression of the soy leghemoglobin apoprotein and the yeast heme B prosthetic group, which combine to form soy leghemoglobin protein. Impossible Foods has sequenced the *P. pastoris* production strain genome, verifying the sequence of the inserted DNA and confirming the production strain does not contain antibiotic resistance genes. Impossible Foods also states that the transformed DNA is stably integrated in the production strain.

Impossible Foods states that soy leghemoglobin preparation is manufactured by submerged batch fed fermentation of the *P. pastoris* production strain under controlled conditions. The culture is periodically tested to ensure production strain identity, purity, and protein generating ability. Following fermentation, the *P. pastoris* cells are lysed by mechanical shearing and the insoluble content is removed by centrifugation and microfiltration. The resulting lysate is concentrated by ultrafiltration, stabilized with sodium chloride and sodium ascorbate, and stored as a frozen liquid concentrate. The frozen concentrate is standardized to a final concentration of 6-9% of soy leghemoglobin protein. Impossible Foods states that the raw materials used in the production of soy leghemoglobin preparation are food grade, that the manufacturing process is performed in accordance with current good manufacturing practices, and that the components of the fermentation media are not derived from major food allergens.

Impossible Foods provides specifications for soy leghemoglobin preparation; these include solids ($\leq 24\%$ w/w), which includes soy leghemoglobin protein content (6-9 %) at a purity of $\geq 65\%$, fat ($\leq 2\%$), carbohydrates ($\leq 4\%$), ash ($\leq 4\%$), pH (6.5-8.5), and lead (< 0.4 mg/kg), as well as limits for microorganisms. Impossible Foods also provides results from batch analyses that demonstrate soy leghemoglobin preparation can be manufactured to meet these specifications. Impossible Foods states that soy leghemoglobin preparation can be stored at -20 °C as a frozen liquid for at least 12 months with no observable change in soy leghemoglobin protein stability.

Impossible Foods estimates dietary exposure to the soy leghemoglobin protein and the soy leghemoglobin preparation at the maximum use level of 0.8% soy leghemoglobin protein. Impossible Foods estimates mean and 90th percentile dietary exposures for the general population based on the conservative assumption that consumers will substitute ground beef analogue products containing soy leghemoglobin preparation for traditional meat products on a 1:1 basis. Impossible Foods used food consumption data from “Retail Commodity Intakes: Mean Amounts of Retail Commodities per Individual” (USDA, 2007-2008). Impossible Foods estimates mean and 90th percentile intake of soy leghemoglobin protein to be 3.3 mg/kg bw/d, and 6.7 mg/kg bw/d respectively.

Impossible Foods also estimates 90th percentile intake of soy leghemoglobin preparation to be 8.9 mg/kg bw/d, accounting for the *P. pastoris* proteins present in the final soy leghemoglobin preparation.

Impossible Foods uses several lines of evidence to develop a weight-of-evidence approach to assess the safety of soy leghemoglobin preparation for use in food. In addition to considering the safety of *P. pastoris* for use as the production microorganism, Impossible Foods considers (1) the history of consumption of hemoglobin proteins in food, (2) the results of bioinformatic analyses comparing soy leghemoglobin and *P. pastoris* proteins to known toxins and allergens, (3) the digestibility of soy leghemoglobin preparation proteins in simulated gastric fluid, and (4) publicly available scientific literature. Impossible Foods also describes publicly available experimental evidence from toxicity studies, along with a detailed discussion of the evidence and its relevance to their safety assessment.

Impossible Foods discusses the prevalence and function of hemoglobin proteins, which are found in the tissues of plants and animals commonly consumed in the human diet. These proteins are involved in selective transport, storage, or buffering of oxygen levels in cells and tissues. Examples of dietary sources of plant-derived hemoglobins include malted grain products and sprouted seeds, grains, rice, and beans.

Impossible Foods assesses the potential for soy leghemoglobin and *P. pastoris* proteins to be toxic or allergenic. Bioinformatic analyses of soy leghemoglobin protein were conducted using both sequence alignment- and Support Vector Machine (SVM)-based methods, while analyses of the 17 most abundant *P. pastoris* proteins were conducted using the sequence alignment-based method alone. Impossible Foods reports the sequence-alignment results demonstrate that neither soy leghemoglobin nor the 17 analyzed *P. pastoris* proteins contain significant amino acid sequence homology to known or putative allergens or toxins. Impossible Foods further reports that the combined results of multiple SVM analyses predict that soy leghemoglobin is not likely to be an allergen. Impossible Foods reports that the digestibility analysis shows that proteins in the soy leghemoglobin preparation are digested by pepsin in simulated gastric fluid. Impossible Foods concludes that soy leghemoglobin and the *P. pastoris* proteins within the preparation have little or no toxic or allergenic potential.

Impossible Foods reports that the published scientific literature was searched for reports of toxicity or allergenicity associated with soy leghemoglobin or with *P. pastoris*. Impossible Foods states that the literature search did not identify information that suggested allergic, toxic, or adverse health effects related to consumption of soy leghemoglobin or *P. pastoris* proteins.

Impossible Foods describes a published study that it conducted. This study includes a bacterial reverse mutation assay and a chromosomal aberration assay in human peripheral blood lymphocytes; these demonstrate soy leghemoglobin preparation is non-mutagenic and non-clastogenic. The published study also includes 14- and 28-day oral toxicity studies in rats; Impossible Foods reports that there were no treatment-related, toxicologically relevant effects up to 1536 mg/kg/day, the highest dose of the soy leghemoglobin preparation tested.

Impossible Foods includes the report of a panel of individuals (Impossible Foods' GRAS panel). Based on its review, Impossible Foods' GRAS panel concluded that soy leghemoglobin preparation is safe under the conditions of its intended use.

Based on the publicly available scientific data assembled and presented in its GRAS notice, Impossible Foods' concludes that soy leghemoglobin preparation is generally recognized as safe for use to optimize flavor in ground beef analogue products intended to be cooked.

Potential Labeling Issues

Under section 403(a) of the Federal Food, Drug, and Cosmetic Act (FD&C Act), a food is misbranded if its labeling is false or misleading in any way. Section 403(r) of the FD&C Act lays out the statutory framework for labeling claims characterizing a nutrient level in a food or the relationship of a nutrient to a disease or health-related condition (also referred to as nutrient content claims and health claims). The notice raises a potential issue under these labeling provisions. In the notice, Impossible Foods states that soy leghemoglobin preparation has nutritive value as a source of iron. If products containing soy leghemoglobin preparation bear any nutrient content or health claims on the label or in labeling, such claims are subject to the applicable requirements and are under the purview of ONFL. OFAS did not consult with ONFL on this issue or evaluate any information in terms of labeling claims. Questions related to food labeling should be directed to ONFL.

Allergen Labeling

The FD&C Act requires that the label of a food that is or contains an ingredient that contains a "major food allergen" declare the allergen's presence (section 403(w)). The FD&C Act defines a "major food allergen" as one of eight foods or food groups (i.e., milk, eggs, fish, Crustacean shellfish, tree nuts, peanuts, wheat, and soybeans) or a food ingredient that contains protein derived from one of those foods. Soy leghemoglobin preparation requires labeling under the FD&C Act because it contains protein derived from soybean.

Potential Requirement for a Color Additive Petition

There is no GRAS provision for color additives. In Impossible Foods' notice, soy leghemoglobin preparation is described as red/brown. As such, the use of soy leghemoglobin preparation in food products (other than ground beef analogue products intended to be cooked) may constitute a color additive use under section 201(t)(1) of the FD&C Act and FDA's implementing regulations in 21 CFR Part 70. Under section 201(t)(1) and 21 CFR 70.3(f), a color additive is a material that is a dye, pigment, or other substance made by a synthetic process or similar artifice, or is extracted, isolated, or otherwise derived from a vegetable, animal, mineral, or other source. Under 21 CFR 70.3(g), a material that otherwise meets the definition of a color additive can be exempt from that definition if it is used (or is intended to be used) solely for a purpose or purposes other than coloring. Our response to GRN 000737 is not an approval for use as

a color additive nor is it a finding of the Secretary of the Department of Health and Human Services within the meaning of section 721(b)(4) of the FD&C Act. Questions about color additives should be directed to the Division of Petition Review in OFAS.

Section 301(ll) of the FD&C Act

Section 301(ll) of the FD&C Act prohibits the introduction or delivery for introduction into interstate commerce of any food that contains a drug approved under section 505 of the FD&C Act, a biological product licensed under section 351 of the Public Health Service Act, or a drug or a biological product for which substantial clinical investigations have been instituted and their existence made public, unless one of the exemptions in section 301(ll)(1)-(4) applies. In our evaluation of Impossible Foods' notice concluding that soy leghemoglobin preparation is GRAS under its intended conditions of use, we did not consider whether section 301(ll) or any of its exemptions apply to foods containing soy leghemoglobin preparation. Accordingly, our response should not be construed to be a statement that foods containing soy leghemoglobin preparation, if introduced or delivered for introduction into interstate commerce, would not violate section 301(ll).

Conclusions

Based on the information that Impossible Foods provided, as well as other information available to FDA, we have no questions at this time regarding Impossible Foods' conclusion that soy leghemoglobin preparation is GRAS under its intended conditions of use to optimize flavor in ground beef analogue products intended to be cooked. This letter is not an affirmation that soy leghemoglobin preparation is GRAS under 21 CFR 170.35. Unless noted above, our review did not address other provisions of the FD&C Act. Food ingredient manufacturers and food producers are responsible for ensuring that marketed products are safe and compliant with all applicable legal and regulatory requirements.

In accordance with 21 CFR 170.275(b)(2), the text of this letter responding to GRN 000737 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

**Dennis M.
Keefe -S**

Digitally signed by Dennis M. Keefe -S
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