Janet E. Collins, Ph.D., R.D.
Motif FoodWorks, Inc.
27 Drydock Avenue, 2nd Floor
Boston, Massachusetts 02210

Re: GRAS Notice No. GRN 001001

Dear Dr. Collins:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 001001. We received Motif FoodWorks, Inc.'s (Motif) notice on April 20, 2021 and filed it on June 28, 2021. Motif submitted amendments to the notice received on August 12, 2021, September 3, 2021, and October 18, 2021 that provided clarification on the identity, manufacturing, specifications, analytical methods, stability data, and estimated dietary exposure.

The subject of the notice is myoglobin preparation from a strain of *Pichia pastoris* expressing the myoglobin gene from *Bos taurus* (myoglobin preparation) at levels up to 2% myoglobin to impart flavor and aroma in ground meat and poultry analogue products. The notice informs us of Motif's view that this use of myoglobin preparation is GRAS through scientific procedures.

Our use of the term "myoglobin 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 (CFSAN). The Office of Food Additive Safety (OFAS) did not consult with ONFL regarding the appropriate common or usual name for "myoglobin preparation."

Motif states that myoglobin is a protein identified by the UniProtKB number P02192 and Gene Identifier 280695 that has an amino acid sequence identical to myoglobin protein from *Bos taurus*. Motif describes myoglobin preparation as a liquid composed of water, myoglobin protein, *P. pastoris* proteins, ash, fat, and carbohydrates. Motif states that myoglobin preparation is formulated with food grade excipients, stabilizers, preservatives (e.g., sodium phosphate, sodium ascorbate, sodium chloride), and antimicrobial agents.

Motif states that the *P. pastoris* production strain t838417, is derived from the parental

strain t303048 via a sequence of transformations to overexpress the myoglobin protein.[1] Motif states that its *P. pastoris* production strain is a non-pathogenic, non-toxigenic, well-characterized methyltrophic yeast with a history of safe use in the food industry. Motif describes the construction of the production strain, using (1) synthetic genes encoding eight enzymes native to *P. pastoris* to overexpress the proteins of the heme biosynthetic pathway, (2) a synthetic gene encoding the myoglobin protein from *Bos taurus* codon-optimized for expression in *P. pastoris*, and (3) an additional copy of the gene encoding a transcription factor native to *P. pastoris* to optimize expression of the promoters. Motif states that the production strain was constructed using principles described by the OECD criteria for Good Industrial Large Scale Practice microorganisms.

Motif states that the myoglobin preparation is manufactured by submerged fed-batch fermentation of the *P. pastoris* production strain. Motif states that the fermentation is carried out under controlled conditions and the production strain is tested to ensure microbial purity, specific growth rate, and yield. After fermentation, the *P. pastoris* cells are washed and lysed by mechanical shearing and the insoluble material is removed by treating the cells with sodium phosphate buffer to clear the cell debris, followed by centrifugation and microfiltration. The resulting lysate is subjected to ultrafiltration to concentrate myoglobin protein. The protein concentrate is stabilized by addition of sodium chloride, sodium phosphate, sodium ascorbate, and other food-grade antimicrobials and antioxidants. Motif states that the final myoglobin preparation is stored at -20 °C as a frozen liquid. Motif states that all raw materials used in the production of myoglobin preparation are food-grade and that the components of the fermentation medium are not derived from major food allergens.

Motif provides specifications for myoglobin preparation that include myoglobin protein (≥ 3%) at a purity of ≥ 65% w/w. Specifications also include total organic solids (≤ 7.5%), moisture (≥ 92.5%), fat (≤ 1%), carbohydrates (≤ 0.5%), ash (≤ 1.5%), pH (6.5 - 8.5), lead (≤ 0.01 mg/kg), as well as limits on microorganisms. Motif provides results from analyses of three batches to demonstrate that myoglobin preparation can be manufactured to meet these specifications.

Motif discusses the stability of the myoglobin preparation and states that the preparation remains as a liquid dispersion of myoglobin protein in water at up to 60 °C. Motif states that the myoglobin protein begins to unfold at 40 °C although it remains suspended. Motif also states that the myoglobin protein changes color as confirmed by UV-vis analysis. This is because the heme iron is reduced from oxymyoglobin to metmyoglobin when heated above at 40 °C. Based on studies that assessed the quality and appearance of raw and cooked foods containing the preparation, Motif estimates the shelf life to be 12 months when stored at -20 °C and ≤7 days when thawed and

[1] Motif states that the host strain of *P. pastoris,* t303048, is deposited in the collection at the Northern Regional Research Laboratories (NRRL) as Y-7556 and is identical to the well-characterized strain Y-11430. Motif notes that *P. pastoris* strain NRRL Y-11430 served as the host organism in the production of soy leghemoglobin in GRN 000737. FDA responded to GRN 000737 on July 23, 2018 and stated that we had no objections to the notifier's GRAS conclusion.

stored under refrigerated conditions (≤8 °C).

Motif states that the intended use of myoglobin preparation is in plant-based ground meat and poultry analogue products providing levels up to 2% myoglobin protein to impart flavor and aroma. Motif states that flavor and aroma of myoglobin preparation mimics those associated with cooked ground meat and provides examples of the intended meat and poultry analogue products to include burgers, patties, sausages, and other plant-based meat analogues, including fresh and/or frozen entrées or meals, where ground meat or poultry is typically the principal ingredient.

Motif provides estimates of dietary exposure to myoglobin protein from dietary sources and from the intended uses of myoglobin preparation. Motif estimates the average *per capita* dietary exposure to myoglobin protein from dietary sources to be approximately 1 g/person (p)/day (d) based on loss-adjusted food availability data for red meat, poultry and fish reported by the U.S. Department of Agriculture Economic Research Service and the mean estimated myoglobin protein concentrations in these foods reported in published literature. Motif states that ground beef and poultry analogue products are substitutional for conventional meat and poultry products. Therefore, Motif concludes that the intended use of myoglobin preparation would not increase the dietary exposure to myoglobin protein in the U.S. population. Motif also provides estimates of dietary exposure to myoglobin protein based on the intended use and consumption data for meat and poultry analogue products from the National Health and Nutrition Examination Survey, 2013-2018. Motif estimates the mean and 90th percentile dietary exposures to myoglobin protein for the U.S. population aged 2 years and older (eaters only) to be 1.43 and 2.86 g/p/d, respectively, based on the maximum use level of myoglobin preparation. Motif states that the typical use levels of myoglobin preparation are 1 to 1.25% and concludes that mean dietary exposure to myoglobin protein from typical use levels to be approximately 1 g/p/d and comparable to background dietary exposure to myoglobin protein from consumption of meat and poultry products. Based on the maximum use level of myoglobin preparation (2% myoglobin), Motif also estimates the mean and 90th percentile dietary exposures to the myoglobin preparation for the U.S. population aged 2 years and older (eaters only) to be 31.70 and 97.56 g/p/d, respectively.

Motif states that the intended use of myoglobin preparation in ground meat and poultry analogue products is self-limiting due to its organoleptic properties that may limit consumer acceptability.

Motif states that myoglobin protein is present in all commonly consumed meat sources, such as beef, pork, and poultry, and has an extensive history of safe consumption by the global population. Motif provides the amino acid sequence of its myoglobin protein, which is identical to that of bovine myoglobin. Motif conducted a comprehensive literature search through March 2021 and did not identify any publication that would contradict its conclusion on the safety of myoglobin consumption.

Motif states that myoglobin protein is not considered one of the major allergens, and bioinformatic analysis showed that bovine myoglobin does not share significant

sequence similarity with known allergens. Motif concludes that bovine myoglobin is unlikely to be an allergen in food and would not pose any significant allergenic risk.

Motif notes that except for the gene encoding bovine myoglobin, the production strain does not contain any other exogenous DNA. Motif states that the lack of toxigenicity of *P. pastoris* proteins has been discussed in the published literature. Since Motif's production strain is derived from a safe host strain lineage that is described in the published literature, Motif concludes that the small concentrations of residual *P. pastoris* proteins present in the ingredient would not pose a safety concern in terms of toxicity or allergenicity.

Motif includes the report of a panel of individuals (Motif's GRAS panel). Based on its review, Motif's GRAS panel concluded that myoglobin preparation is safe under the conditions of its intended use.

Based on the totality of safety data and information, Motif concludes that its myoglobin preparation is safe and GRAS for the intended use.

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. Motif states that myoglobin preparation contains iron. If products containing myoglobin 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 in CFSAN. 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.

Potential Requirement for a Color Additive Petition

There is no GRAS provision for color additives. In the notice, Motif notes that although the primary function of the myoglobin preparation is to impart flavor, myoglobin imparts a red coloration when exposed to oxygen in some food applications. As such, the use of myoglobin preparation in food products 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 001001 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 Food Ingredients 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 Motif's notice concluding that myoglobin 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 myoglobin preparation. Accordingly, our response should not be construed to be a statement that foods containing myoglobin preparation, if introduced or delivered for introduction into interstate commerce, would not violate section 301(ll).

Conclusions

Based on the information that Motif provided, as well as other information available to FDA, we have no questions at this time regarding Motif's conclusion that myoglobin preparation is GRAS under its intended conditions of use. This letter is not an affirmation that myoglobin 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 001001 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J. Carlson -S

Digitally signed by Susan J. Carlson -S
Date: 2021.12.03 09:59:41 -05'00'

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Director
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