U.S. FOOD & DRUG ADMINISTRATION
Human Foods Program

Aletta Schnitzler, Ph.D.
TurtleTree Labs, Inc.
1100 Main Street
Suite 300
Woodland, CA 95695

Re: GRAS Notice No. GRN 001219

Dear Dr. Schnitzler:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 001219. We received TurtleTree Labs, Inc.'s (TurtleTree) notice on September 30, 2024, and filed it on December 4, 2024. TurtleTree submitted amendments to the notice on February 14, 2025, and April 1, 2025, that clarified the identity, intended use, manufacturing, specifications, dietary exposure, and aspects of the safety narrative.

The subject of the notice is recombinant bovine lactoferrin isolate produced by *Komagataella phaffii* "M020" expressing the gene encoding bovine lactoferrin (rbLf isolate) for use as an ingredient in chewing gum at levels up to 3%; dairy and non-dairy ice cream and frozen desserts at levels up to 0.2%; non-dairy milk at levels up to 0.04%; powdered milk at levels up to 0.4%; nutrition bars, nutritional beverages and protein and nutritional powders at levels ranging from 0.04 to 0.25%; and dairy and non-dairy yogurt at levels up to 0.1%. The notice informs us of TurtleTree's view that these uses of rbLf isolate are GRAS through scientific procedures.

TurtleTree provides information on the identity and composition of rbLf isolate, describing it as an off-white, light tan to light pink, or salmon-colored powder containing ≥80% protein (of which ≥95% is full-length rbLf), carbohydrates, fat, ash, and moisture. bLf is an iron-binding glycoprotein of 689 amino acids, has a molecular weight of 80-87 kilodaltons, and is designated by CAS Registry Number 14897-68-9. TurtleTree states that rbLf is substantially similar in structure and function to bLf.

TurtleTree describes the production organism used in the manufacture of rbLf isolate, stating that *K. phaffii* is non-pathogenic and non-toxigenic and that the introduced DNA in the production strain does not encode for toxins or allergens. The production strain, *K. phaffii* "M020," was created though the genetic modification of parental strain *K. phaffii* "YB-4290," including insertions and deletions of several genes to express and secrete rbLf at high levels. TurtleTree describes *K. phaffii* "M020" as containing two copies of a gene encoding for bLf. Additionally, the DNA sequence of bLf has been codon optimized for expression in *K. phaffii*, while conserving the native amino acid sequence of bLf. TurtleTree states that the production strain does not contain any antibiotic

resistance genes, and the stability of the genetic modification has been confirmed by sequencing.

TurtleTree states that rbLf isolate is manufactured through submerged fed-batch fermentation of the production organism, *K. phaffii* "M020," under controlled conditions. After fermentation, the biomass is removed from the fermentation broth via centrifugation followed by microfiltration. The filtrate containing rbLf isolate is concentrated, the pH is optionally adjusted, and the filtrate is subjected to ion-exchange chromatography. The eluted fraction, enriched with rbLf isolate, is further concentrated through ultrafiltration and diafiltration to remove salts and impurities. The concentrated rbLf-buffered solution is then spray dried to obtain the final rbLf isolate. TurtleTree states that rbLf isolate is manufactured in accordance with current good manufacturing practices and that all raw materials and processing aids are food grade and are used in accordance with applicable U.S. regulations or are GRAS for their intended uses. TurtleTree states that none of the materials used in the production of rbLf isolate are derived from major food allergens.

TurtleTree provides specifications for rbLf isolate that include protein content (≥80% w/w, of which ≥95% (w/w) is full-length rbLf), carbohydrate (≤5% w/w), fat (<2% w/w), water activity (<0.6 aw), ash (≤9.5% w/w), moisture (≤8.5% w/w), pH (5.6-7.2), iron (≤120 mg/100 g), and limits for heavy metals, including lead (≤0.05 mg/kg) and microorganisms, such as *Salmonella* serovars (absent in 25 g) and *Listeria monocytogenes* (absent in 25 g). TurtleTree provides the results from three non-consecutive batch analyses to demonstrate that rbLf isolate can be manufactured to meet these specifications. TurtleTree states that rbLf is stable for 24 months at <30°C and moderate humidity (<75%) when stored unopened in its original packaging.

TurtleTree estimates the dietary exposure to rbLf based on the intended uses of rbLf and food consumption data from the 2017-2020 National Health and Nutrition Examination Survey (NHANES). TurtleTree estimates the mean and 90th percentile eaters-only dietary exposures to rbLf isolate for the U.S. population aged 2 years and older to be 155 mg/person (p)/d (2.7 mg/kg body weight (bw)/d) and 321 mg/p/d (5.8 mg/kg bw/d), respectively.[1] TurtleTree states that intended uses of rbLf isolate are substitutional to the current uses of bLf and are expanded to include non-dairy analogs of certain foods; therefore, there will be no substantial increase in the cumulative dietary exposure to bLf from the intended uses.

TurtleTree discusses the publicly available data and information supporting the safety of rbLf isolate, which includes a summary of similarities and differences between rbLf isolate and bLf, a discussion of published toxicological and clinical studies with bLf, and a summary supporting the safety of the production organism.

TurtleTree states that the safety of rbLf isolate is supported by a long history of safely consuming bLf and notes that rbLf isolate shares the same sequence as the published

[1] We estimated the mean and 90th percentile eaters-only dietary exposures to rbLf isolate for the U.S. population aged 2 years and older to be 177 mg/p/d (3.0 mg/kg bw/d) and 375 mg/p/d (6.5 mg/kg bw/d), respectively, using food consumption data from the 2017-2020 NHANES.

reference sequence for bLf. TurtleTree summarizes a published study comparing the structure and function of rbLf isolate to bLf and concludes they are substantially similar to each other. The authors of the study note minor differences, including slight changes in the glycan profile and a higher degree of iron saturation in rbLf isolate compared to bLf. Despite the higher degree of iron saturation, the authors demonstrate that rbLf isolate has similar iron binding and release compared to bLf. TurtleTree also considers daily iron exposure from the intended uses of rbLf isolate coupled with background iron intake and concludes that daily dietary exposure levels to iron would be well below the National Academy of Medicine's tolerable upper intake levels across all age subpopulations. Additionally, TurtleTree discusses the absorption, distribution, metabolism, excretion, and digestion through oral exposure to bLf, noting a slight decrease in rbLf isolate digestibility compared to bLf. However, TurtleTree concludes that the observed minor differences in structure and function between rbLf isolate and bLf do not pose a safety concern.

TurtleTree incorporates into the notice and summarizes toxicological studies on bLf from GRN 000464.[2] These include a published 13-week oral toxicity study in rats; unpublished acute, 4-week, and chronic oral toxicity studies in rats; and an unpublished genotoxicity study. TurtleTree notes that no test-article related effects were observed in these toxicity studies, and bLf was found to be non-genotoxic. To further support safety, TurtleTree summarizes clinical studies where bLf was consumed by toddlers, children, and adults. No adverse effects were observed in these studies.

TurtleTree states that the production organism, *K. phaffii,* has a long history of safe use in food production. TurtleTree incorporates into the notice the relevant safety data and studies on *K. phaffii* from GRN 000737.[3] TurtleTree discusses the potential for allergic reactions to any residual proteins from *K. phaffii* and concludes that the low concentration of these proteins remaining in rbLf isolate does not pose a safety or allergenic concern. TurtleTree states that rbLf isolate could elicit an allergic reaction in consumers with a milk allergy; however, as the amino acid sequence of rbLf isolate is identical to bLf, TurtleTree concludes there is no additional risk of allergic response compared to bLf. TurtleTree reports than an updated literature search did not yield new data or information that would contradict TurtleTree's GRAS conclusion.

Based on the totality of the data and information, TurtleTree concludes that rbLf isolate is GRAS for its intended use.

Standards of Identity

In the notice, TurtleTree states its intention to use rbLf isolate in several food categories, including foods for which standards of identity exist, located in Title 21 of the CFR. We

[2] Cow's milk-derived lactoferrin is the subject of GRN 000464. We evaluated this notice and responded in a letter dated February 18, 2014, stating that we had no questions at that time regarding the notifier's GRAS conclusion.

[3] Soy leghemoglobin preparation from a strain of *Pichia pastoris* is the subject of GRN 000737. We evaluated this notice and responded in a letter dated July 23, 2018, stating that we had no questions at that time regarding the notifier's GRAS conclusion.

note that an ingredient that is lawfully added to food products may be used in a standardized food only if it is permitted by the applicable standard of identity.

Potential Labeling Issues

Under section 403(a) of the Federal Food, Drug, & Cosmetic (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). If products containing rbLf isolate 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 the Office of Nutrition and Food Labeling (ONFL) in the Nutrition Center of Excellence. The Office of Pre-Market Additive Safety (OPMAS) 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 nine foods or food groups (i.e., milk, eggs, fish, Crustacean shellfish, tree nuts, peanuts, wheat, soybeans, and sesame) or a food ingredient that contains protein derived from one of those foods. rbLf isolate requires labeling under the FD&C Act because it is a milk protein.

Potential Requirement for a Color Additive Petition

There is no GRAS provision for color additives. In the notice, TurtleTree describes rbLf isolate as an off-white, light tan to light pink, or salmon-colored powder. As such, the use of rbLf isolate 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 001219 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 OPMAS.

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 TurtleTree's notice concluding that rbLf isolate 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 rbLf isolate. Accordingly, our response should not be construed to be a statement that foods containing rbLf isolate, if introduced or delivered for introduction into interstate commerce, would not violate section 301(ll).

Conclusions

Based on the information that TurtleTree provided, as well as other information available to FDA, we have no questions at this time regarding TurtleTree's conclusion that rbLf isolate is GRAS under its intended conditions of use. This letter is not an affirmation that rbLf isolate 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 001219 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J. Carlson -S

Digitally signed by Susan J. Carlson -S
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Director
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