U.S. FOOD & DRUG ADMINISTRATION
Human Foods Program

Wing Yu
CIRS Group USA, Inc.
4250 Fairfax Drive, Suite 600
Arlington, VA 22203

Re: GRAS Notice No. GRN 001203

Dear Ms. Yu:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 001203. We received the notice that you submitted on behalf of Guilin Layn Natural Ingredients Corp. (Layn) on June 14, 2024 and filed it on September 16, 2024. Layn submitted an amendment to the notice on November 12, 2024 that explained the generation of the gene insertion in the production organism, provided additional information on the identity of the notified substance, and confirmed the regulatory status of a substance used in the manufacturing process.

The subject of the notice is rebaudioside M2 obtained by enzymatic treatment of steviol glycosides purified from the leaves of *Stevia rebaudiana* (Bertoni) Bertoni (rebaudioside M2) for use as a general-purpose sweetener in foods, excluding infant formula and products under the U.S. Department of Agriculture's jurisdiction, at levels determined by good manufacturing practices. The notice informs us of Layn's view that these uses of rebaudioside M2 are GRAS through scientific procedures.

The rebaudioside M2 that is the subject of GRN 001203 is made from highly purified components of the leaves of the stevia plant. We note that a GRAS notice for the use of specific purified components of stevia, such as rebaudioside M2, and FDA's response do not necessarily apply to the uses of other stevia products.

Our use of the terms "rebaudioside M2," "steviol glycosides," or "SGs" in this letter is not our recommendation of these terms as appropriate common or usual names 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 Nutrition Center for Excellence. The Office of Pre-Market Additive Safety did not consult with ONFL regarding the appropriate common or usual names for "rebaudioside M2, "steviol glycosides," and "SGs."

Layn provides information about the identity and composition of rebaudioside M2. Layn states that the subject of the notice is >75% rebaudioside M2 with minor amounts of

rebaudioside D, rebaudioside D2, rebaudioside M8, rebaudioside A, and rebaudioside B. Rebaudioside M2 (CAS Reg. No. 1638974-11-4) is a glycoside of steviol and is one of a group of known SGs, which differ from each other by the number of glycoside moieties and bonding order.

Layn describes the production organisms and method of manufacture used in the production of rebaudioside M2. The process uses non-pathogenic and non-toxicogenic strains of *Escherichia coli* (derived from *E. coli* BL21 *lacZY*) that are engineered to express a uridine-5′-diphospho-(UDP) glucosyltransferase that catalyzes the conversion of steviol glycosides to rebaudioside M2 and a sucrose synthase that catalyzes the conversion of UDP to UDP-glucose. Layn provides information on the parent strain, *E. coli* BL21(DE3), and describes the genes used to express the enzymes. Layn states that the enzymes produced are not likely to be allergenic and that *E. coli* BL21(DE3) has a history of safe use as a production source for food ingredients. The production organisms are grown in a culture medium, and the fermentation culture is subsequently filtered, homogenized, diluted, and centrifuged to remove the cell biomass. The filtrate containing the expressed enzymes is mixed with a solution containing an extract of the leaves of *S. rebaudiana* that contains ≥95% of total SGs and is primarily rebaudioside A.[1] The reaction results in the conversion of rebaudioside A to rebaudioside M2. The reaction is terminated by heat treatment that inactivates the enzymes. The resulting solution is then decolorized by membrane filtration and treatment with an ion exchange resin. The solution is then subjected to a microporous adsorption resin to retain SGs, which are subsequently eluted with ethanol. The solution is concentrated by evaporation, decolorized with activated carbon, filtered, concentrated, and finally spray-dried to obtain the final rebaudioside M2. Layn states that rebaudioside M2 is produced under current good manufacturing practices and that all raw materials, processing aids, and food contact substances used to manufacture rebaudioside M2 are food grade and are used in accordance with U.S. regulations or are GRAS for their respective uses.

Layn provides specifications for rebaudioside M2 that include the content of total SGs (≥ 95 %, dry matter basis (DM)), rebaudioside M2 (≥ 75 %, DM), limits for total ash (≤ 1 %), loss on drying (≤ 6 %), lead (≤ 1 mg/kg), arsenic (≤ 1 mg/kg), methanol (≤ 200 mg/kg), ethanol (≤ 5000 mg/kg), and limits on microorganisms. Layn provides results from the analyses of three non-consecutive batches to demonstrate that rebaudioside M2 can be produced in accordance with these specifications.

Layn provides an estimate of dietary exposure to rebaudioside M2. Layn discusses a published study on dietary exposures to rebaudioside A (Ref. 1). Based on the methodology described in Ref. 1 and a relative sweetness intensity as low as 250 times that of sucrose, Layn estimates maximum dietary exposure in adults (expressed as steviol equivalents) to be 0.90 mg/kg body weight (bw)/day (d) and in children to be 0.99 mg/kg bw/d. Layn states that the use of rebaudioside M2 in food is self-limiting due to organoleptic factors and consumer taste considerations.

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[1] Layn notes that the starting material, rebaudioside A purified from the leaves of a *S. rebaudiana*, is the subject of GRN 000354. We evaluated this notice and responded in a letter dated July 15, 2011, stating that we had no questions at that time regarding the notifier's GRAS conclusion.

Layn summarizes published studies pertaining to the metabolic fate and safety of rebaudioside M2. Based on the pharmacokinetic studies, Layn concludes that microbes in the colon hydrolyze SGs completely to steviol and thus rebaudioside M2 shares a common metabolic fate. Layn discusses previously reviewed published acute, subchronic, and chronic toxicity/carcinogenicity studies, published multi-generational reproductive and developmental toxicology studies conducted with rebaudioside A, and *in vitro* and *in vivo* mutagenicity/genotoxicity studies for the safety conclusion for rebaudioside M2. Layn includes an update of the literature regarding the safety of SGs through March 2024 and reports that no studies relevant to toxicology were found that would alter its safety conclusion.

To further support its view that rebaudioside M2 is GRAS for the intended use, Layn summarizes the decisions on the safety of SGs by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), the European Food Safety Authority, Food Standards Australia New Zealand, and Health Canada for use in food as sweeteners. Layn notes that JECFA has established an acceptable daily intake (ADI) for SGs of 0-4 mg/kg bw/d (expressed as steviol equivalents). This ADI was based on a no observed adverse effect level of 970 mg/kg bw/d (383 mg/kg bw/d, as steviol equivalents) from a two-year rat study, and the application of a safety factor of 100 to account for intra- and inter-species differences.

Based on all the available scientific information, Layn concludes that rebaudioside M2 is GRAS for its intended use in foods.

Standards of Identity

In the notice, Layn states its intention to use rebaudioside M2 in several food categories, including foods for which standards of identity exist, located in Title 21 of the CFR. We 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.

Section 301(ll) of the Federal Food, Drug, and Cosmetic Act (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 Layn's notice concluding that rebaudioside M2 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 rebaudioside M2. Accordingly, our response should not be construed to be a statement that foods containing rebaudioside M2, if introduced or delivered for introduction into interstate commerce, would not violate section 301(ll).

Conclusions

Based on the information that Layn provided, as well as other information available to FDA, we have no questions at this time regarding Layn's conclusion that rebaudioside M2 is GRAS under its intended conditions of use. This letter is not an affirmation that rebaudioside M2 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 001203 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J. Carlson -S

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Susan J. Carlson, Ph.D.
Director
Division of Food Ingredients
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