Martin Hahn
Hogan Lovells US LLP
555 Thirteenth Street, NW,
Washington, DC 20004

Re: GRAS Notice No. GRN 001106

Dear Mr. Hahn:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 001106. We received the notice that you submitted on behalf of Manus Bio, Inc. (Manus Bio) on September 23, 2022, and filed it on February 9, 2023. We received amendments to the notice on February 7, 2023, and March 30, 2023, confirming the status of the notifier's initial agent and information about the production method.

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

The rebaudioside I that is the subject of GRN 001106 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 I, and FDA's response do not necessarily apply to the uses of other stevia products.

Our use of the terms "rebaudioside I," and "steviol glycosides," or "SGs" in this letter is not our recommendation of these terms 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 did not consult with ONFL regarding the appropriate common or usual name for "rebaudioside I" and "SGs."

Manus Bio provides information about the identity and composition of rebaudioside I. Rebaudioside I (CAS Reg. No. 1220616-34-1), a glycoside of steviol, is identified as (4-α-13-[(*O*-β-D-glucopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranosyl)oxy]-kaur-16-en-18-oic acid 3-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl ester. Rebaudioside I is one of a group of known SGs, which differ from each other by the number of glycoside moieties and bonding order.

Manus Bio describes the production organism used in the manufacture of rebaudioside I. The process uses a non-pathogenic and non-toxicogenic strain of *Escherichia coli* (derived from *E. coli* K-12 sub-strain MG1655 Fnr-) that is engineered to express a uridine-5′-diphospho-(UDP) glucosyltransferase that catalyzes the conversion of SGs to rebaudioside I and a sucrose synthase that catalyzes the conversion of UDP to UDP-glucose.

Manus Bio provides information about the method of manufacture of rebaudioside I and states that all raw materials and processing aids used are food grade and meet applicable regulations. The manufacturing process starts with a purified extract of the leaves of *S. rebaudiana* (Bertoni) Bertoni (stevia extract). Manus Bio states that the stevia extract, containing ≥ 90% SGs, is obtained from stevia leaves by a series of steps, including crushing, dissolution, solvent extraction, and precipitation. Manus Bio notes that the manufacturing process for the stevia extract is described in detail in GRN 000275.[1] Rebaudioside I is produced from the stevia extract using the production organism. For this step, the *E. coli* strain is grown in culture medium containing the stevia extract, and the UDP-glucosyltransferases catalyze the glycosylation of SGs to rebaudioside I. After sufficient rebaudioside I is produced, the media and *E. coli* biomass are inactivated by heating. The mixture is subjected to centrifugation and filtration to remove the biomass and precipitated enzymes. Rebaudioside I is then obtained by a series of steps including filtration, aqueous crystallization, centrifugation, rinsing and drying. Manus Bio states that the dried rebaudioside I may be crystalline or amorphous and may be further milled, spray-dried, freeze-dried, agglomerated, compacted, and granulated or other physical modifications to obtain the desirable particle size of the final product.

Manus Bio provides specifications for rebaudioside I that include the content of total SGs (≥ 95 %), rebaudioside I (≥ 95 %), limits for total ash (≤ 1 %), loss on drying (≤6 %), lead (≤ 1 mg/kg), arsenic (≤ 1 mg/kg), mercury (≤ 1 mg/kg), cadmium (≤ 1 mg/kg), methanol (≤ 200 mg/kg), ethanol (≤ 5000 mg/kg), and limits on microorganisms. Manus Bio provides results from three, non-consecutive batch analyses to demonstrate that rebaudioside I can be produced in accordance with the specifications.

Manus Bio provides an estimate of dietary exposure to rebaudioside I. Manus Bio 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 167

[1] The subject of GRN 000275 is purified steviol glycosides with rebaudioside A as the principal component that is obtained from the leaves of *S. rebaudiana* (Bertoni) Bertoni for use as a tabletop sweetener. We responded to this notice in a letter dated June 11, 2009, stating that we did not have any questions regarding the notifier's GRAS conclusion.

times that of sucrose, Manus Bio estimates maximum dietary exposure in adults (expressed as steviol equivalents) to be 1.51 mg/kg body weight (bw)/day (d) and in children to be 1.67 mg/kg bw/d. Manus Bio states that the use of rebaudioside I in food is self-limiting due to organoleptic factors and consumer taste considerations.

Manus Bio summarizes published studies pertaining to the metabolic fate and safety of rebaudioside I. Based on the pharmacokinetic studies, Manus Bio concludes that microbes in the colon hydrolyze SGs completely to steviol and thus rebaudioside I shares a common metabolic fate. Manus Bio 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 I. Manus Bio includes an update of the literature regarding the safety of SGs through January 2022 and reports that no studies relevant to toxicology were found that would alter its safety conclusion.

To further support its view that rebaudioside I is GRAS for the intended use, Manus Bio 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. Manus Bio 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, Manus Bio concludes that rebaudioside I is GRAS for its intended use in foods.

Standards of Identity

In the notice, Manus Bio states its intention to use rebaudioside I in several food categories, including foods for which standards of identity exist, located in Title 21 of the Code of Federal Regulations. 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 its review of Manus Bio's notice that rebaudioside I is GRAS for the intended use, FDA did not consider whether section 301(ll) or any of its exemptions apply to foods containing rebaudioside I. Accordingly, this response should

not be construed to be a statement that foods that contain rebaudioside I, if introduced or delivered for introduction into interstate commerce, would not violate section 301(ll).

Conclusions

Based on the information that Manus Bio provided, as well as other information available to FDA, we have no questions at this time regarding Manus Bio's conclusion that rebaudioside I is GRAS under its intended conditions of use. This letter is not an affirmation that rebaudioside I 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 001106 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J. Carlson -S

Digitally signed by Susan J. Carlson -S
Date: 2023.06.16 15:23:23 -04'00'

Susan Carlson, Ph.D.
Director
Division of Food Ingredients
Office of Food Additive Safety
Center for Food Safety
and Applied Nutrition

Reference

1. Renwick, A.G. 2008. The use of a sweetener substitution method to predict dietary exposures for the intense sweetener rebaudioside A. Food and Chemical Toxicology 46:S61–S69.