Jun Hua
Sichuan Ingia Biosynthetic Co., Ltd.
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Chengdu, Sichuan Province
CHINA

Re: GRAS Notice No. GRN 001178

Dear Mr. Hua:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 001178. We received the notice that you submitted on behalf of Sichuan Ingia Biosynethetic Co. Ltd. (SIB) on January 25, 2024, and filed it on April 17, 2024. We received amendments on June 12, 2024, and June 21, 2024, that provide clarifying information on the composition and batch analyses of rebaudioside I.

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 SIB's view that these uses of rebaudioside I are GRAS through scientific procedures.

The rebaudioside I that is the subject of GRN 001178 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," "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."

SIB 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.

SIB 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* BL21 (DE3)) that is engineered to express a uridine-5′-diphospho-(UDP) glucosyltransferase that catalyzes the conversion of steviol glycosides to rebaudioside I and a sucrose synthase that catalyzes the conversion of UDP to UDP-glucose. The production organism is grown in a culture medium, and the expression of the glucosyltransferase and sucrose synthase enzymes is induced by the addition of isopropyl β-D-thiogalactopyranoside. The fermentation culture is centrifuged to separate and concentrate the production organism cell biomass. The biomass 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. The reaction results in the conversion of rebaudioside A to rebaudioside I. The reaction is terminated by the addition of water and citric acid with heat that inactivates and denatures the enzymes. The resulting solution undergoes filtration and crystallization steps to obtain the rebaudioside I product. SIB states that the mother liquor remaining from the crystallization step may be subjected to a macroporous resin that retains remaining rebaudioside I, which is then eluted with ethanol. The ethanol is removed by evaporation and the rebaudioside I product is obtained by drying.

SIB 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. SIB provides results from the analyses of ten batches, including five non-consecutive batches, to demonstrate that rebaudioside I can be produced in accordance with the stated specifications.

SIB provides an estimate of dietary exposure to rebaudioside I. SIB 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 170 times that of sucrose, SIB estimates maximum dietary exposure in adults (expressed as steviol equivalents) to be 1.49 mg/kg body weight (bw)/day (d) and in children to be 1.64 mg/kg bw/d. SIB states that the use of rebaudioside I in food is self-limiting due to organoleptic factors and consumer taste considerations.

SIB summarizes published studies pertaining to the metabolic fate and safety of rebaudioside I. Based on the pharmacokinetic studies, SIB concludes that microbes in the colon hydrolyze SGs completely to steviol and thus rebaudioside I shares a common metabolic fate. SIB 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. SIB includes an update of the literature regarding the safety of SGs through December 2023 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, SIB 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. SIB 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, SIB concludes that rebaudioside I is GRAS for its intended use in foods.

Standards of Identity

In the notice, SIB 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 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 SIB'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 SIB provided, as well as other information available to FDA, we have no questions at this time regarding SIB'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 001178 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J. Carlson -S

Digitally signed by Susan J. Carlson -S
Date: 2024.08.16 13:37:39 -04'00'

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