



Lei Zhang
Regulatory Affairs Manager
Sichuan Ingia Biosynthetic Co., Ltd.
2-1111#, No. 368 Tianfu 2nd Street, Hi-tech Zone
Chengdu, Sichuan, 610041
CHINA

Re: GRAS Notice No. GRN 001184

Dear Mr. Zhang:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 001184. We received the notice that you submitted on behalf of Sichuan Ingia Biosynthetic Co. Ltd. (SIB) on March 25, 2024, and filed it on June 3, 2024. We received amendments on August 22, 2024, and September 5, 2024, that updated the contact person information and provided information regarding residual kaurenoic acid.

The subject of the notice is rebaudioside M from a modified strain of *Escherichia coli* BL21(DE3) (rebaudioside M) 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 SIB's view that the use of rebaudioside M is GRAS through scientific procedures.

The rebaudioside M that is the subject of GRN 001184 is made from a modified strain of *E. coli* BL21(DE3). We note that a GRAS notice for the use of specific purified components of stevia, such as rebaudioside M, and FDA's response do not necessarily apply to the uses of other stevia products.

Our use of the terms "rebaudioside M," "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 M," "steviol glycosides," and "SGs."

SIB provides information about the identity and composition of rebaudioside M. SIB states that the notified substance is a white to yellow powder that is $\geq 95\%$ rebaudioside

M and <5% of the sum of stevioside, rebaudioside D, and rebaudioside A. Rebaudioside M (CAS No. 1220616-44-3) 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.

SIB provides information about the manufacturing process for rebaudioside M. Rebaudioside M is produced via a strain of *E. coli* BL21(DE3) engineered to express enzymes used in the production of SGs. SIB provides information on the parent strain, *E. coli* BL21(DE3), and describes the genes used to express the enzymes that encompass the production pathway of SGs (e.g., rebaudioside M). SIB states that the production strain is neither toxigenic nor pathogenic, and the enzymes produced are not likely to be allergenic, induce antibiotic resistance or virulence, and that *E. coli* BL21(DE3) has a history of safe use as a production source for food ingredients.

The manufacturing process begins with the fermentation of the *E. coli* production strain under aerobic conditions. Expression of the recombinant enzymes is induced by the addition of isopropyl- β -D-thiogalactopyranoside. SGs are produced through a series of enzymatic reactions resulting in production of rebaudioside M. The enzymatic reaction is terminated by heat treatment and acidification in which viable cells are killed and enzymes are denatured and inactivated. The resulting solution is filtered to obtain a crude SG product that contains rebaudioside M. The crude product is subjected to a series of purification and concentration steps, including a macroporous resin that retains rebaudioside M. The rebaudioside M is then eluted with ethanol. The combined eluate is concentrated by evaporation followed by a crystallization step using ethanol. The resulting crystals are separated by filtration and the filtrate (residual mother liquor) is retained. The crystals are resuspended with ethanol and activated carbon, filtered, and rebaudioside M is obtained by recrystallization. The rebaudioside M crystals are separated by filtration, and the second filtrate is also retained. The retained filtrates are subjected to a macroporous resin column that retains the remaining rebaudioside M, which is then eluted with ethanol. The eluate is processed as previously described. The wet rebaudioside M crystals are dried, crushed, sieved, and mixed to obtain the final rebaudioside M product.

SIB provides specifications for rebaudioside M that include the content of total SGs ($\geq 95\%$ on a dry-weight basis) and rebaudioside M ($\geq 95\%$ on a dry-weight basis). Specifications also include limits for kaurenoic acid (< 0.3 mg/kg), ash ($\leq 1\%$), loss on drying ($\leq 6\%$), lead (≤ 0.1 mg/kg), arsenic (≤ 0.1 mg/kg), mercury (≤ 0.1 mg/kg), cadmium (≤ 0.1 mg/kg), methanol (≤ 200 mg/kg), ethanol (≤ 1000 mg/kg), as well as limits on microorganisms. SIB provides results from the analyses of five non-consecutive batches to demonstrate that rebaudioside M can be produced in accordance with the stated specifications.

SIB discusses the results of stability studies conducted with rebaudioside M. Rebaudioside M was stored under ambient conditions (25 °C and 60 % relative humidity) for up to 24 months and under accelerated conditions (40 °C and 75 % relative humidity) for up to 6 months. SIB states that rebaudioside M met all specification parameters throughout the study period and concludes that rebaudioside M is stable under the conditions tested.

SIB provides an estimate of dietary exposure to rebaudioside M. 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 of 300 times that of sucrose, SIB estimates maximum dietary exposure in adults (expressed as steviol equivalents) to be 0.74 mg/kg body weight (bw)/d and in children to be 0.81 mg/kg bw/d. SIB states that the use of rebaudioside M 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 M. Based on the pharmacokinetic studies, SIB concludes that microbes in the colon hydrolyze SGs completely to steviol and thus rebaudioside M 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 M. SIB 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 M 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 M is GRAS for its intended use in foods.

Standards of Identity

In the notice, SIB states its intention to use rebaudioside M 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(II) of the Federal Food, Drug, and Cosmetic Act (FD&C Act)

Section 301(II) 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(l)(1)-(4) applies. In our evaluation of SIB's notice concluding that rebaudioside M is GRAS under its intended conditions of use, we did not consider whether section 301(l) or any of its exemptions apply to foods containing rebaudioside M. Accordingly, our response should not be construed to be a statement that foods that contain rebaudioside M, if introduced or delivered for introduction into interstate commerce, would not violate section 301(l).

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 M is GRAS under its intended conditions of use. This letter is not an affirmation that rebaudioside M 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 001184 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

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Mical E. Honigfort -S
Date: 2024.10.23
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for Susan Carlson, Ph.D.

Director

Division of Food Ingredients

Office of Pre-Market Additive Safety

Office of Food Chemical Safety, Dietary

Supplements, and Innovation

Human Foods Program

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.