Hua Jun
President
Sichuan Ingia Biosynthetic Co., Ltd.
Room 7-701#, Tongwei International Centre, No., 588
Central Tianfu Avenue, High-tech Zone
Chengdu, Sichuan Province
CHINA

Re: GRAS Notice No. GRN 000764

Dear Mr. Jun:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 000764. We received Sichuan Ingia Biosynthetic Co., Ltd.'s (Sichuan Ingia) notice on February 27, 2018, and filed it on March 29, 2018. Sichuan Ingia submitted amendments to the notice on April 25, 2018, and May 6, 2018, that provide additional information about the method of manufacture.

The subject of the notice is rebaudioside D obtained by yeast-mediated enzyme modification of rebaudioside A purified from the leaves of *Stevia rebaudiana* (Bertoni) Bertoni (rebaudioside D) 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 Sichuan Ingia's view that this use of rebaudioside D is GRAS, through scientific procedures.

The rebaudioside D that is the subject of GRN 000764 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 D, and FDA's response do not necessarily apply to the uses of other stevia products.

Our use of the terms "rebaudioside D obtained by yeast-mediated enzyme modification of rebaudioside A," "rebaudioside D," or "SGs" in this letter is not our recommendation of those 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 D."

Sichuan Ingia provides information about the identity and composition of rebaudioside D. Rebaudioside D (CAS No. 63279-13-0), a glycoside of steviol, is identified as 13-[(2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, 2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl-β-D-glucopyranosyl ester. Rebaudioside D is one of a group of known steviol glycosides (SGs), which differ from each other by the number of glycoside moieties and bonding order.

Sichuan Ingia describes the method of manufacture of rebaudioside D. The manufacturing process starts with the production of a purified extract of *S. rebaudiana* (stevia). Stevia leaves are extracted in hot water, filtered, and concentrated. The extract is subjected to an adsorption resin that is then eluted with methanol. The eluate is deionized using an ion exchange resin, concentrated, and dried. The dried extract is dissolved in aqueous ethanol, filtered, and crystallized. The crystals are separated, rinsed with ethanol, and recrystallized. Sichuan Ingia states that the resulting product is ≥ 95% rebaudioside A. Next, a non-pathogenic and non-toxicogenic strain of *Pichia pastoris* (derived from *P. pastoris* ATCC 20864) expressing a uridine-5′-diphospho-(UDP) glucosyltransferase is used to catalyze the conversion of rebaudioside A to rebaudioside D. The *P. pastoris* strain is grown in culture medium and the cells harvested by filtration. The cells are suspended in a buffer and combined with the rebaudioside A extract and the reaction allowed to proceed. The resultant mixture is centrifuged and the supernatant is heated to denature any residual enzyme and kill any remaining yeast cells. The supernatant is filtered and subjected to an adsorption resin that is then eluted with ethanol. The eluate is concentrated by evaporation and cooled to crystallize. The wet crystals are washed, dissolved in ethanol, treated with activated charcoal, and rebaudioside D is recrystallized and dried.

Sichuan Ingia provides specifications for rebaudioside D that include the content of rebaudioside D (≥ 95 %), as well as limits for total ash (≤ 1 %), loss on drying (≤ 6 %), lead (< 0.05 mg/kg), arsenic (< 0.05 mg/kg), mercury (< 0.05 mg/kg), cadmium (< 0.05 mg/kg), methanol (< 200 mg/kg), ethanol (< 1000 mg/kg), as well as limits on microorganisms. Sichuan Ingia provides results from five, non-consecutive batch analyses to demonstrate that rebaudioside D can be produced to meet specifications.

Sichuan Ingia provides estimates of dietary exposure to rebaudioside D. Sichuan Ingia 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 250 times that of sucrose, Sichuan Ingia reports the maximum dietary exposure in adults (expressed as steviol equivalents) to be 0.91 mg/kg body weight (bw)/day (d) and in children to be 1.01 mg/kg bw/d. Sichuan Ingia states that the use of rebaudioside D in food is self-limiting due to organoleptic factors and consumer taste considerations.

Sichuan Ingia summarizes published studies pertaining to the metabolic fate and safety of rebaudioside D. Based on pharmacokinetic studies, Sichuan Ingia concludes that microbes in the colon hydrolyze SGs completely to steviol and thus rebaudioside D

shares a common metabolic fate. Sichuan Ingia discusses previously reviewed published acute, subchronic, and chronic toxicity/carcinogenicity studies; published multi-generational reproductive and developmental toxicology studies conducted with rebaudioside A as well as *in vitro* and *in vivo* mutagenicity/genotoxicity studies for the safety conclusion of rebaudioside D. Sichuan Ingia includes an update of the literature regarding the safety of rebaudioside D through December 2017 and reports that no studies relevant to toxicology were found that would alter its safety conclusion.

To further support its view that rebaudioside D is GRAS for the intended use, Sichuan Ingia 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. Sichuan Ingia 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.

Sichuan Ingia includes the statement of a panel of individuals (Sichuan Ingia's GRAS panel). Based on its review, Sichuan Ingia's GRAS panel concluded that rebaudioside D is safe under the conditions of its intended use.

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

Standards of Identity

In the notice, Sichuan Ingia states its intention to use rebaudioside D 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 Sichuan Ingia's notice that rebaudioside D is GRAS for the intended use, FDA did not consider whether section 301(ll) or any of its exemptions apply to foods containing rebaudioside D. Accordingly, this response should not be construed to be a statement that foods that contain rebaudioside D, if introduced or delivered for introduction into interstate commerce, would not violate section 301(ll).

Conclusions

Based on the information that Sichuan Ingia provided, as well as other information available to FDA, we have no questions at this time regarding Sichuan Ingia's conclusion that rebaudioside D is GRAS under its intended conditions of use. This letter is not an affirmation that rebaudioside D 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 000764 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Michael A. Adams -S

Digitally signed by Michael A. Adams -S
Date: 2018.07.10 13:07:19 -04'00'

Dennis M. Keefe, Ph.D.
Director
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.