William J. Rowe
President
GRAS Associates, LLC
27499 Riverview Center Blvd., Suite 212
Bonita Springs, FL 34134

Re: GRAS Notice No. GRN 000823

Dear Mr. Rowe:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 000823. We received the notice that you submitted on behalf of Blue California on November 27, 2018, and filed it on January 29, 2019.

The subject of the notice is rebaudioside E obtained by enzyme treatment of steviol glycosides (SGs) purified from the leaves of *Stevia rebaudiana* (Bertoni) Bertoni (rebaudioside E) for use as a general purpose sweetener in foods, other than infant formula and meat and poultry products, in accordance with current good manufacturing practices (cGMP), as well as use as a table top sweetener. The notice informs us of Blue California's view that these uses of rebaudioside E are GRAS through scientific procedures.

The rebaudioside E that is the subject of GRN 000823 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 E, and FDA's response do not necessarily apply to the uses of other stevia products.

Our use of the terms "rebaudioside E," "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 E."

Blue California provides information about the identity and composition of rebaudioside

E. Rebaudioside E (CAS Reg. No. 63279-14-1), a glycoside of steviol, is identified as 13-[(2-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy] ent-kaur-16-en-19-oic acid-(2-O-β-D-glucopyranosyl-β-D-glucopyranosyl) ester. Rebaudioside E is one of a group of known SGs, which differ from each other by the number of glycoside moieties and bonding order.

Blue California describes the production strain used in the manufacture of rebaudioside E. The process uses a non-pathogenic and non-toxicogenic strain of *Pichia pastoris* (derived from *P. pastoris* ATCC 20864) expressing a uridine-5′-diphospho-(UDP) glucosyltransferase that catalyzes the conversion of stevioside to rebaudioside E and a sucrose synthase that catalyzes the conversion of UDP to UDP-glucose.

Blue California provides information about the method of manufacture of rebaudioside E and states that all reagents, solvents, and processing aids used are food grade and meet applicable regulations. The manufacturing process starts with the production of a purified extract of *S. rebaudiana* (stevia extract). Stevia leaves are extracted with either hot water or aqueous ethanol and the extract is then subjected to an adsorption resin that retains SGs. The SGs are eluted from the resin with ethanol and then recrystallized. The SGs are collected by filtration and then spray-dried. Blue California states that the dried extract contains ≥ 95% total SGs. Rebaudioside E is produced from the stevia extract using a preparation of *P. pastoris*. For this step, the *P. pastoris* strain is grown in culture medium, harvested by centrifugation, and the enzymes separated by homogenization and centrifugation. The enzyme fraction is then re-suspended in a reaction buffer and the mixture combined with the stevia extract to allow the enzymes to catalyze their relevant reactions. The resultant mixture is heated to denature the enzymes, and then filtered. The resulting solution is subjected to an adsorption resin that retains rebaudioside E. The resin is washed with buffer and the rebaudioside E is then eluted with ethanol. The eluate is concentrated by evaporation and then cooled to allow rebaudioside E to crystallize and precipitate. The wet crystals are collected, washed, and dissolved in ethanol. The solution is treated with activated charcoal, and rebaudioside E is recrystallized, collected by filtration, and dried to yield the final rebaudioside E product.

Blue California provides specifications for rebaudioside E that include the content of total SGs (≥ 95 %) and rebaudioside E (≥ 85 %), limits for total ash (≤ 1 %), loss on drying (≤ 5 %), lead (< 0.5 mg/kg), arsenic (< 0.5 mg/kg), mercury (< 0.5 mg/kg), cadmium (< 0.5 mg/kg), methanol (< 200 mg/kg), ethanol (< 1000 mg/kg), as well as limits on microorganisms. Blue California provides results from five, non-consecutive batch analyses to demonstrate that rebaudioside E can be produced in accordance with the specifications.

Blue California provides an estimate of dietary exposure to rebaudioside E. Blue California 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 137 times that of sucrose, Blue California estimates maximum dietary exposure in adults (expressed as steviol equivalents) to be 2.15 mg/kg body weight (bw)/day (d) and in children to be 2.38 mg/kg bw/d. Blue California states that the use of rebaudioside E

in food is self-limiting due to organoleptic factors and consumer taste considerations.

Blue California summarizes published studies pertaining to the metabolic fate and safety of rebaudioside E. Based on the pharmacokinetic studies, Blue California concludes that microbes in the colon hydrolyze SGs completely to steviol and thus rebaudioside E shares a common metabolic fate. Blue California 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 E. Blue California includes an update of the literature regarding the safety of SGs through September 2018 and reports that no studies relevant to toxicology were found that would alter its safety conclusion.

To further support its view that rebaudioside E is GRAS for the intended use, Blue California 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. Blue California 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.

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

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

Standards of Identity

In the notice, Blue California states its intention to use rebaudioside E 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 Blue California's notice that rebaudioside E is GRAS for the intended use, FDA did not consider whether section 301(ll) or any of its

exemptions apply to foods containing rebaudioside E. Accordingly, this response should not be construed to be a statement that foods that contain rebaudioside E, if introduced or delivered for introduction into interstate commerce, would not violate section 301(ll).

Conclusions

Based on the information that Blue California provided, as well as other information available to FDA, we have no questions at this time regarding Blue California's conclusion that rebaudioside E is GRAS under its intended conditions of use. This letter is not an affirmation that rebaudioside E 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 000823 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J. Carlson -S

Digitally signed by Susan J. Carlson -S
Date: 2019.06.21 16:27:34 -04'00'

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