Katrina Emmel, Ph.D.
GRAS Associates, LLC
27499 Riverview Center Blvd., Suite 212
Bonita Springs, FL 34134

Re: GRAS Notice No. GRN 000667

Dear Dr. Emmel:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 000667. We received the notice, dated August 9, 2016, that you submitted on behalf of Blue California, in accordance with the agency's proposed regulation, proposed 21 CFR 170.36 (62 FR 18938; April 17, 1997; Substances Generally Recognized as Safe (GRAS); the GRAS proposal) on August 17, 2016, and filed it on September 14, 2016. We received amendments providing clarification about information provided in the notice as well as composition, specifications, method of manufacture, starting material, source organism and the enzymes involved in the manufacture on November 8, 2016, December 9, 2016, and December 12, 2016.

FDA published the GRAS final rule on August 17, 2016 (81 FR 54960), with an effective date of October 17, 2016. As GRN 000667 was pending on the effective date of the GRAS final rule, we requested some additional information consistent with the format and requirements of the final rule. We received an amendment responding to this request on October 20, 2016.

The subject of the notice is rebaudioside M. The notice informs FDA of the view of Blue California that rebaudioside M is GRAS, through scientific procedures, for use as a general purpose sweetener in foods, other than infant formula and meat and poultry products, in accordance with good manufacturing practices, as well as use as a table top sweetener.

The rebaudioside M that is the subject of GRN 000667 is made from steviol glycosides (SGs) as defined by international standards. We note that a GRAS notice for the use of a specific substance and FDA's response do not necessarily apply to the uses of other stevia products.

Our use of "rebaudioside M" in this letter is not our recommendation of that term 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. We did not consult with ONFL regarding the appropriate common or usual name for "rebaudioside M."

U.S. Food & Drug Administration
Center for Food Safety & Applied Nutrition
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Blue California provides information about the identity and composition of rebaudioside M. Blue California describes rebaudioside M as a white powder that contains ≥ 95% rebaudioside M (CAS Reg. No. 1220616-44-3). Rebaudioside M is one of a group of known steviol glycosides (SGs), which differ from each other by the number of glycoside moieties and bonding order. Blue California notes that rebaudioside M includes a steviol backbone with two 2-O-ß-D-glucopyranosyl-3-O-ß-D-glucopyranosyl-ß-D-glucopyranosyl units, an ether at position C-13 and an ester at position C-19.

Blue California describes the production strains used in the manufacture of rebaudioside M. The process uses two non-pathogenic and non-toxicogenic strains of *Pichia pastoris*. *P. pastoris* strain A expresses a uridine 5′-diphospho-(UDP) glucosyltransferase that catalyzes the conversion of stevioside to rebaudioside E. *P. pastoris* strain B expresses a UDP-glucosyltransferase that catalyzes the conversion of rebaudioside E to rebaudioside M and a sucrose synthase that catalyzes the conversion of UDP to UDP-glucose.

Blue California provides information about the method of manufacture of rebaudioside M 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 *Stevia rebaudiana* (Bertoni) Bertoni (stevia extract). Stevia leaves are extracted in water and the extract is then subjected to an adsorption resin that retains SGs. The SGs are eluted from the resin with ethanol, and the eluate is then filtered and spray-dried. Blue California states that the powdered extract contains ≥ 95% total SGs. The purified SGs product is then converted to rebaudioside M using *P. pastoris* strains A and B. For this step, the two *P. pastoris* strains are grown separately in culture seed media, harvested by centrifugation, and then re-suspended in a reaction buffer. The two *P. pastoris* cell suspensions are mixed together with the stevia extract to allow the enzymes to catalyze their relevant reactions. The resultant mixture is centrifuged to remove the *P. pastoris* cells from the supernatant that contains rebaudioside M. The supernatant is filtered to remove residual cell debris and then subjected to an adsorption resin that retains rebaudioside M. The resin is washed with buffer and the rebaudioside M is then eluted with ethanol. The eluate is concentrated by evaporation and then cooled to allow rebaudioside M to crystallize and precipitate. The wet crystals are collected, washed, and dissolved in ethanol. The solution is treated with activated charcoal, and rebaudioside M is re-crystallized, dried, and sifted to yield the final rebaudioside M product.

Blue California provides specifications for rebaudioside M that include the content of rebaudioside M (≥ 95%), as well as limits for total ash (≤ 1%), loss on drying (≤ 6%), lead (< 0.5 mg/kg), arsenic (< 0.5 mg/kg), methanol (< 200 mg/kg), ethanol (< 1000 mg/kg), and limits on microbial contaminants. Although rebaudioside M is not one of the nine SGs listed in the specifications for SGs established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) at its 73rd meeting in June 2010, Blue California concludes that rebaudioside M has a similar structure to other SGs and therefore adopted specifications for rebaudioside M that are consistent with those established for steviol glycosides by JECFA. Blue California provides results from five, non-consecutive batch analyses of rebaudioside M that demonstrate compliance with these specifications.

Blue California provides an estimate of dietary exposure to rebaudioside M resulting from its intended use in foods. Blue California utilized the methodology reported in GRN 000301, where the notifier used estimated dietary intake and body weight data for sweeteners from a published study on dietary exposures to rebaudioside A (Ref. 1). In this study, published data on intake of intense sweeteners were converted to sucrose equivalents using estimates of sweetness relative to sucrose. Blue California determined the relative sweetness of rebaudioside M to be

200 times that of sucrose. Based on this sweetness intensity factor, Blue California estimates the maximum dietary exposure in adults (expressed as steviol equivalents) to be 1.7 mg/kg body weight per day (bw/d) and in children to be 1.88 mg/kg bw/d. Blue California states that the use of rebaudioside M in food is self-limiting due to organoleptic factors and consumer taste considerations.

Blue California discusses published and unpublished studies pertaining to the metabolic fate and safety of rebaudioside M. Based on these studies, Blue California concludes that the final metabolic fate of rebaudioside M is the same as naturally occurring SGs. Blue California discusses published acute, subchronic, and chronic studies as well as published mutagenicity/genotoxicity, reproduction and developmental studies, in relation to their safety determination for rebaudioside M. Blue California includes an update of the literature for the time period through June 2016 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, Blue California summarizes recent decisions by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), the Food Standards Australia New Zealand (FSANZ), the European Food Safety Authority (EFSA), and other governmental bodies on the safety of SGs for use in food as a sweetener. Blue California notes that JECFA established an acceptable daily intake for SGs of 0-4 mg/kg bw/d (expressed as steviol). This ADI was based on a no observed adverse effect level of 970 mg/kg bw/d (383 mg/kg bw/d, as steviol) from a two year rat study, and the application of a one hundred-fold safety factor 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 M is safe under the conditions of its intended use.

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 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 000667 is accessible to the public at www.fda.gov/grasnoticeinventory.

Standards of Identity

In the notice, Blue California 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 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 M is GRAS for the intended use, FDA did not consider whether section 301(ll) or any of its exemptions apply to foods containing rebaudioside M. Accordingly, this 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(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 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 000667 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Michael A. Adams -S

Digitally signed by Michael A. Adams -S
DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=1300042713, cn=Michael A. Adams -S
Date: 2017.02.17 17:07:42 -05'00'

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.