Evangelia C. Pelonis
Keller and Heckman, LLP
1001 G Street, N.W. Suite 500W
Washington, D.C. 20005

Re: GRAS Notice No. GRN 000812

Dear Ms. Pelonis:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 000812. We received the notice you submitted on behalf of Amyris, Inc. (Amyris) on September 3, 2018, and filed it on October 16, 2018.

The subject of the notice is rebaudioside M from *Saccharomyces cerevisiae* (rebaudioside M) 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 Amyris' view that this use of rebaudioside M is GRAS, through scientific procedures.

Our use of the terms "rebaudioside M from *Saccharomyces cerevisiae*," "rebaudioside M," "steviol glycosides," or "SGs" 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. The Office of Food Additive Safety did not consult with ONFL regarding the appropriate common or usual name for "rebaudioside M."

Amyris provides information about the identity and composition of rebaudioside M. Rebaudioside M (CAS No. 1220616-44-3), 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 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.

Amyris provides information about the manufacturing process for rebaudioside M. Rebaudioside M is produced via a daughter strain of *S. cerevisiae* CEN.PK113-7D engineered to express enzymes used in the production of SGs. Amyris provides information on the genetics of the parent strain of *S. cerevisiae* and describes the genes used to express the enzymes that encompass the production pathway of SGs (e.g., rebaudioside M). Amyris states that the production strain is neither toxigenic nor pathogenic, contains no antibiotic resistance genes, and that *S. cerevisiae* has a history of safe use as a production source for food ingredients. The manufacturing process begins with the fermentation of cane sugar using the *S. cerevisiae* production strain. Amyris states that rebaudioside M is biosynthesized and excreted during the fermentation. Following fermentation, the production organism is removed by centrifugation and the supernatant is sterilized and filtered. The supernatant is subsequently subjected to an adsorption resin that is then eluted with ethanol. The ethanol extract is concentrated by evaporation, filtered, and crystallized to obtain rebaudioside M.

Amyris provides specifications for rebaudioside M that include the content of total SGs (≥ 95%), rebaudioside M (≥ 95 %). Specifications also include limits for total ash (≤ 1%), loss on drying (≤ 5%), lead (< 1 mg/kg), arsenic (< 1 mg/kg), mercury (< 1 mg/kg), cobalt (< 0.03 mg/kg), cadmium (< 1 mg/kg), methanol (< 200 mg/kg), ethanol (< 3000 mg/kg), as well as limits on microorganisms. Amyris provides results from three, non-consecutive batch analyses to demonstrate that rebaudioside M can be produced to meet specifications.

Amyris provides estimates of dietary exposure to rebaudioside M. Amyris 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, Amyris estimates maximum dietary exposure in adults (expressed as steviol equivalents) to be 0.90 mg/kg body weight (bw)/day (d) and in children to be 0.99 mg/kg bw/d. Amyris states that the use of rebaudioside M in food is self-limiting due to organoleptic factors and consumer taste considerations.

Amyris summarizes published studies pertaining to the metabolic fate and safety of rebaudioside M. Based on pharmacokinetic studies, Amyris concludes that microorganisms in the colon hydrolyze SGs completely to steviol and thus rebaudioside M shares a common metabolic fate. Amyris discusses previously reviewed published acute, subchronic, and chronic toxicity/carcinogenicity studies; published multigenerational reproductive and developmental toxicology studies conducted with rebaudioside A; as well as *in vitro* and *in vivo* mutagenicity/genotoxicity studies for its safety conclusion of rebaudioside M. Amyris includes an update of the literature regarding the safety of rebaudioside M through August 2018 and reports that no studies relevant to safety/toxicology were found that would alter its safety conclusion.

To further support its view that rebaudioside M is GRAS for the intended use, Amyris 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.

Amyris notes that JECFA 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.

Amyris includes the report of a panel of individuals (Amyris' GRAS panel). Based on its review, Amyris' GRAS panel concluded that rebaudioside M is safe under the conditions of its intended use.

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

Standards of Identity

In the notice, Amyris 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 Amyris' 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 Amyris provided, as well as other information available to FDA, we have no questions at this time regarding Amyris' 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 000812 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Dennis M. Keefe -S

Digitally signed by Dennis M. Keefe -S
Date: 2018.12.08 08:41:43 -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.