



Katherine Vega, Ph.D.
DSM Nutritional Products
45 Waterview Boulevard
Parsippany, NJ 07054

Re: GRAS Notice No. GRN 000759

Dear Dr. Vega:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 000759. We received DSM's notice on January 18, 2018, and filed it on February 28, 2018.

The subject of the notice is rebaudioside M from *Yarrowia lipolytica* (rebaudioside M) for use as a flavor and 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 DSM's view that this use of rebaudioside M is GRAS, through scientific procedures.

Our use of the terms "rebaudioside M from *Yarrowia lipolytica*," "rebaudioside M" 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."

DSM 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.

DSM provides information about the manufacturing process for rebaudioside M. Rebaudioside M is produced *via* a high SG producing daughter strain of *Y. lipolytica*

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obtained after the mating of two parent *Y. lipolytica* strains that were engineered to produce SGs. DSM provides information on the genetics of the parent strains of *Y. lipolytica* and describes the genes¹ used to express the enzymes that encompass the production pathway of SGs (e.g., rebaudioside M). DSM states that the production strain is neither toxigenic nor pathogenic and that *Y. lipolytica* has a history of safe use as a production source for food ingredients. DSM states that the production strain contains no residual antibiotic resistance genes and is susceptible to antifungal agents. DSM uses a controlled, submerged, aerobic fed batch fermentation process with the *Y. lipolytica* production strain. DSM states that rebaudioside M is biosynthesized and excreted during the fermentation. Following fermentation, the production organism is removed by centrifugation and the supernatant is heat-treated to inactivate any remaining microorganisms. The supernatant is subsequently clarified by centrifugation and filtration and then concentrated by evaporation. The concentrate is subjected to two crystallization steps and the rebaudioside M crystals are then separated and dried.

DSM provides specifications for rebaudioside M that include the minimum content of rebaudioside M ($\geq 95\%$) and total SGs ($> 95\%$). Specifications also include limits for moisture ($\leq 10\%$), ash ($\leq 1\%$), lead (≤ 1 mg/kg), arsenic (≤ 1 mg/kg), cadmium (≤ 1 mg/kg), mercury (≤ 1 mg/kg), and specified limits for microbial contaminants. DSM provides the results of five batch analyses to demonstrate that rebaudioside M can be produced in accordance with these specifications.

DSM provides estimates of dietary exposure to rebaudioside M. DSM 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 200 times that of sucrose, DSM reports the maximum dietary exposure in adults (expressed as steviol equivalents) to be 1.1 mg/kg body weight/day (bw/d) and in children to be 1.2 mg/kg bw/d. DSM states that the use of rebaudioside M in food is self-limiting due to organoleptic factors and consumer taste considerations.

DSM summarizes published studies pertaining to the metabolic fate and safety of rebaudioside M. Based on pharmacokinetic studies, DSM concludes that microbes in the colon hydrolyze SGs completely to steviol and thus rebaudioside M shares a common metabolic fate. DSM 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 M. DSM includes an update of the literature regarding the safety of SGs 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 M is GRAS for the intended use, DSM 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.

¹ DSM uses *de novo* synthesized, codon optimized for *Y. lipolytica*, genes of the steviol glycoside metabolic pathway from *Stevia rebaudiana* (Bertoni) Bertoni for production of SGs.

DSM 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.

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

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

Standards of Identity

In the notice, DSM 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(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(II)(1)-(4) applies. In its review of DSM's notice that rebaudioside M is GRAS for the intended use, FDA did not consider whether section 301(II) 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(II).

Conclusions

Based on the information that DSM provided, as well as other information available to FDA, we have no questions at this time regarding DSM'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 000759 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,
**Michael A.
Adams -S**

Digitally signed by Michael
A. Adams -S
Date: 2018.05.25 13:29:54
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Dennis M. Keefe, Ph.D.
Director
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.