



Kyra Hjelle
Hjelle Advisors, LLC
1100 Corporate Square Drive
Saint Louis, MO 63132

Re: GRAS Notice No. GRN 001206

Dear Ms. Hjelle:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 001206. We received the notice that you submitted on behalf of Adorvia Biotechnology Co., Ltd. (Adorvia) (Adorvia) on July 16, 2024 and filed it on October 2, 2024. Adorvia submitted an amendment to the notice on January 29, 2025 that provided additional information on the specifications.

The subject of the notice is rebaudioside M produced by enzymatic treatment of rebaudioside A purified from the leaves of *Stevia rebaudiana* (Bertoni) Bertoni (rebaudioside M) for use as a general-purpose sweetener in foods, excluding infant formula and products under the U.S. Department of Agriculture's jurisdiction, at levels determined by good manufacturing practices. The notice informs us of Adorvia's view that these uses of rebaudioside M are GRAS through scientific procedures.

The rebaudioside M that is the subject of GRN 001206 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 M, and FDA's response do not necessarily apply to the uses of other stevia products.

Our use of the terms "rebaudioside M," "steviol glycosides," or "SGs" in this letter is not our recommendation of these terms as appropriate common or usual names 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 Nutrition Center of Excellence. The Office of Pre-Market Additive Safety did not consult with ONFL regarding the appropriate common or usual names for "rebaudioside M," "steviol glycosides," and "SGs."

Adorvia provides information about the identity and composition of rebaudioside M. Adorvia states that the subject of the notice is $\geq 95\%$ total steviol glycosides with $> 50\%$ rebaudioside M and minor amounts of rebaudioside B, rebaudioside I, and rebaudioside 1h. Rebaudioside M (CAS No. 1220616-44-3) is a glycoside of steviol and is one of a

group of known SGs, which differ from each other by the number of glycoside moieties and bonding order.

Adorvia describes the production organisms used in the manufacture of rebaudioside M. The process uses non-pathogenic and non-toxicogenic strains of *Escherichia coli* (derived from *E. coli* BL21 (DE3)) that are engineered to express β -1,2-glucosyltransferase, β -1,3-glucosyltransferase, and sucrose synthase enzymes that are used to catalyze the conversion of steviol glycosides to rebaudioside M. Adorvia provides information on the parent strain, *E. coli* BL21(DE3), and describes the genes used to express the enzymes. Adorvia states that the enzymes produced are not likely to be allergenic and that *E. coli* BL21(DE3) has a history of safe use as a production source for food ingredients. The production organisms are grown in a culture medium, and the expression of the recombinant enzymes is induced by the addition of isopropyl- β -D-thiogalactopyranoside. After the fermentation step is complete, the media is cooled and centrifuged to obtain the microbial biomass. The biomass is mixed with phosphate buffer solution and then homogenized to induce cell lysis. The mixture is transferred to a flocculation tank and subjected to a celite plate and membrane filter to remove cell debris and impurities and obtain a solubilized enzyme preparation. The enzyme preparation is mixed with an extract of the leaves of *S. rebaudiana* that contains >95% SGs and is primarily rebaudioside A as well as sucrose as a source of glucose and either uridine diphosphate or adenosine diphosphate. The reaction results in the conversion of rebaudioside A to rebaudioside M. The reaction mixture is cooled and filtered, and the resulting filter cake is suspended in hot water and filtered. The filter cake is then mixed with ethanol and heated to inactivate and denature the residual enzyme, followed by the addition of water. Activated carbon is added and the mixture filtered. The filtrate is cooled to form rebaudioside M crystals, which are then separated by centrifugation. The product is then dried under vacuum and then pulverized and screened to obtain the final rebaudioside M product.

Adorvia provides specifications for rebaudioside M that include the content of total SGs ($\geq 95\%$, dry matter basis (DM)), rebaudioside M ($> 50\%$ DM), limits for ash ($\leq 1\%$), loss on drying ($\leq 6\%$), lead (≤ 1 mg/kg), arsenic (≤ 1 mg/kg), cadmium (≤ 1 mg/kg), mercury (≤ 1 mg/kg), methanol (≤ 200 mg/kg), ethanol (≤ 5000 mg/kg), and limits on microorganisms. Adorvia provides results from the analyses of five non-consecutive batches to demonstrate that rebaudioside M can be produced in accordance with the stated specifications.

Adorvia provides an estimate of dietary exposure to rebaudioside M. Adorvia 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 250 times that of sucrose, Adorvia 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. Adorvia states that the use of rebaudioside M in food is self-limiting due to organoleptic factors and consumer taste considerations.

Adorvia summarizes published studies pertaining to the metabolic fate and safety of rebaudioside M. Based on the pharmacokinetic studies, Adorvia concludes that

microbes in the colon hydrolyze SGs completely to steviol and thus rebaudioside M shares a common metabolic fate. Adorvia discusses previously reviewed published acute, subchronic, and chronic toxicity/carcinogenicity studies, published multi-generational reproductive and developmental toxicology studies conducted with rebaudioside A, and *in vitro* and *in vivo* mutagenicity/genotoxicity studies for the safety conclusion for rebaudioside M. Adorvia includes an update of the literature regarding the safety of SGs through June 2024 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, Adorvia 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. Adorvia 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.

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

Standards of Identity

In the notice, Adorvia 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 CFR. 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 our evaluation of Adorvia's notice concluding that rebaudioside M is GRAS under its intended conditions of use, we did not consider whether section 301(ll) or any of its exemptions apply to foods containing rebaudioside M. Accordingly, our response should not be construed to be a statement that foods containing rebaudioside M, if introduced or delivered for introduction into interstate commerce, would not violate section 301(ll).

Conclusions

Based on the information that Adorvia provided, as well as other information available to FDA, we have no questions at this time regarding Adorvia'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 001206 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J. Carlson
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Digitally signed by Susan J. Carlson -S
Date: 2025.02.28 16:50:25 -05'00'

Susan J. Carlson, Ph.D.
Director
Division of Food Ingredients
Office of Pre-Market Additive Safety
Office of Food Chemical Safety, Dietary
Supplements, and Innovation
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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.