Sidd Purkayastha, Ph.D.
PureCircle, Ltd.
915 Harger Road, Suite 250
Oak Brook, IL 60523

Re: GRAS Notice No. GRN 000745

Dear Dr. Purkayastha:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 000745. We received PureCircle, Limited's (PureCircle) notice on November 8, 2017, and filed it on December 11, 2017. PureCircle submitted an amendment to the notice on February 13, 2017 that specifies the date of the updated literature review.

The subject of the notice is enzyme modified steviol glycosides (EMSG) obtained by enzyme treatment of steviol glycosides (SGs) purified from the leaves of stevia (*Stevia rebaudiana* (Bertoni) Bertoni) for use as a general purpose sweetener in foods, excluding infant formula and meat and poultry products, at levels determined by current good manufacturing practices, as well as use as a table top sweetener. The notice informs FDA of the view of PureCircle that EMSG is GRAS, through scientific procedures.

The EMSG that is the subject of GRN 000745 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 EMSG, and FDA's response do not necessarily apply to the uses of other stevia products.

Our use of the terms "enzyme modified steviol glycosides," "EMSG" 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 "EMSG."

PureCircle provides information about the identity and composition of EMSG. PureCircle describes SGs as a white to off-white powder that contains ≥ 95% steviol glycosides, and is primarily rebaudioside M. Rebaudioside M (CAS Reg. No. 1220616-44-3) is one of a group of known SGs, which differ from each other by the number of glycoside moieties and bonding order.

PureCircle describes the method of manufacture of EMSG. *S. rebaudiana* leaves are extracted in hot water, and the extract is then filtered, treated with calcium hydroxide, and filtered to remove precipitates. The filtrate is deionized using ion-exchange resins and then subjected to an adsorption resin that retains SGs. The SGs are eluted from the resin with ethanol. The resulting solution is treated with activated carbon, filtered, and the ethanol removed by evaporation. The remaining aqueous solution is deionized with ion-exchange resins, concentrated, and spray dried. The dried powder is dissolved in ethanol and SGs crystallized. The crystals are separated by centrifugation and dried under vacuum. The crystallized product is a minimum of 95% SGs preparation with rebaudioside A as the primary component. Next, *Escherichia coli* LE1B109[1] that carries an expression vector for uridine diphosphate (UDP)-glucosyltransferases and sucrose synthase is grown in culture. The biomass is separated from the culture broth by centrifugation and/or filtration. The biomass is homogenized to disrupt cells, treated with a nuclease, and then filtered to obtain an enzyme preparation. The enzyme preparation and UDP disodium salt are combined with an aqueous solution of the SGs preparation and sucrose to form a reaction mixture. PureCircle states that the reaction results in the conversion of rebaudioside A to rebaudioside M. The mixture is incubated and when the reaction is complete, the mixture is heated to inactivate the enzymes. The resulting mixture is treated with calcium hydroxide and filtered. The filtrate is deionized with ion exchange resins and then subjected to an adsorption resin that retains SGs. The adsorption resin is washed with water and the SGs are then eluted with aqueous ethanol. The SGs are crystallized and separated by centrifugation and dried under vacuum yielding the final EMSG product.

PureCircle provides specifications for EMSG that include minimum content of total SGs (≥ 95%) and limits for total ash (≤ 1%), loss on drying (≤ 6%), lead (< 1 mg/kg), arsenic (< 1 mg/kg), cadmium (< 1 mg/kg), mercury (< 1 mg/kg), methanol (< 0.02%), ethanol (< 0.3%), and specified limits for microbial contaminants. PureCircle provides results of three, non-consecutive batch analyses to demonstrate that EMSG can be produced in accordance with these specifications.

PureCircle provides estimates of dietary exposures to EMSG. PureCircle discusses a published study on dietary exposures to rebaudioside A (Ref. 1), and based on the methodology described in Ref.1 and a relative sweetness intensity of 200 times that of sucrose, PureCircle reports the maximum dietary exposure (expressed as steviol equivalents) in adults to be 1.12 mg/kg body weight (bw)/day and in children to be 1.24 mg/kg body weight (bw)/day. PureCircle states that the use of EMSG in food is self-limiting due to organoleptic factors and consumer taste considerations.

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[1] PureCircle states that *E. coli* LE1B109 is a non-pathogenic and non-toxicogenic derivative of *E. coli* K-12 W3110.

PureCircle summarizes published studies pertaining to the metabolic fate and safety of SGs. Based on the pharmacokinetic studies, PureCircle concludes that microbes in the colon hydrolyze SGs completely to steviol and thus EMSG shares a common metabolic fate. PureCircle 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 EMSG. PureCircle includes an update of the literature regarding the safety of SGs through September 2017 and reports that no studies relevant to toxicology were found that would alter its safety conclusion.

To further support its view that EMSG is GRAS for the intended use, PureCircle 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. PureCircle 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.

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

Based on all the available scientific information, PureCircle concludes that SGs is GRAS for its intended use in foods.

Standards of Identity

In the notice, PureCircle states its intention to use EMSG 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 PureCircle's notice that EMSG is GRAS for the intended use, FDA did not consider whether section 301(ll) or any of its exemptions apply to foods containing EMSG. Accordingly, this response should not be construed to

be a statement that foods that contain EMSG, if introduced or delivered for introduction into interstate commerce, would not violate section 301(ll).

Conclusions

Based on the information that PureCircle provided, as well as other information available to FDA, we have no questions at this time regarding PureCircle's conclusion that EMSG is GRAS under its intended conditions of use. This letter is not an affirmation that EMSG 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 000745 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Michael A. Adams -S

Digitally signed by Michael A. Adams -S
Date: 2018.04.20 16:20:22 -04'00'

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.