

Food Standards Australia New Zealand
PO Box 5423
Kingston, ACT
Australia

Electronic Submission, Application A1157

Enzymatic Production of Reb M

Tate & Lyle is a manufacturer and distributor of rebaudioside M (Reb M) and is making this submission to assist Food Standards Australia New Zealand, FSANZ, in the assessment of novel methods of manufacture for this low calorie sweetener. Tate & Lyle has developed a similar enzymatic conversion method to the one submitted in the Blue California application that results in high purity Reb M.

Tate & Lyle's Reb M is produced by the enzymatic conversion of steviol glycosides using two glucosyltransferase and a sucrose synthase derived from a genetically modified *E. coli* K-12. These enzymes are used to convert steviol glycosides to Reb M in a multistep process, which is detailed in GRAS Notification 780, submitted to FDA on April 27, 2018. Tate & Lyle received a letter of no questions dated July 31, 2018 ([link below](#)). The submission document is attached to this request as it is not posted on the FDA GRAS website at this date.

Additionally, the production strains used to generate the glycosyltransferases and sucrose synthase produced from the recipient strain derived from *E. coli* K-12 are safe for their intended use. Neither the production strain nor the enzymes are present in the final product. Details of the safety evaluation are also included in GRAS Notification 780 (attached).

Therefore, Tate & Lyle requests that Reb M, made by this this novel production method, be included as a permitted sweetener with the same prescribed limits and conditions of use as the application from Blue California (Application A1157).

TATE & LYLE

https://www.accessdata.fda.gov/scripts/fdcc/?set=GRASNotices&id=780&sort=GRN_No&order=DESC&startrow=1&type=basic&search=rebaudioside%20M

8/7/2017

External use permitted

GRAS NOTICE FOR HIGH-PURITY REBAUDIOSIDE M

Prepared for:

Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5001 Campus Drive
College Park, MD
20740 USA

Date:

27 April 2018

GRAS Notice for High-Purity Rebaudioside M

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GRAS Notice for High-Purity Rebaudioside M

Part 1. §170.225 Signed Statements and Certification

In accordance with 21 CFR §170 Subpart E consisting of §170.203 through 170.285, Tate & Lyle hereby informs the United States (U.S.) Food and Drug Administration (FDA) that the high-purity rebaudioside M (≥85% rebaudioside M), manufactured by Tate & Lyle, is not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act based on Tate & Lyle's view that the notified substance is Generally Recognized as Safe (GRAS) under the conditions of its intended use described in Section 1.3 below. In addition, as a responsible official of Tate & Lyle, the undersigned hereby certifies that all data and information presented in this notice represents a complete, representative, and balanced submission, and considered all unfavorable as well as favorable information known to Tate & Lyle and pertinent to the evaluation of the safety and GRAS status of high-purity rebaudioside M (≥85% rebaudioside M) as a general purpose sweetener, as described herein.

Signed,

April 27, 2018

Date

1.1 Name and Address of Notifier

Tate & Lyle
5450 Prairie Stone Parkway
Hoffman Estates, IL
60192 USA

1.2 Common Name of Notified Substance

Rebaudioside M; Reb M; Steviol glycosides; TASTEVA® M

1.3 Conditions of Use

Tate & Lyle intends to market high-purity rebaudioside M, a steviol glycoside preparation comprised of ≥85% rebaudioside M and ≥95% total steviol glycosides, for use as a general purpose sweetener in the U.S., in accordance with current Good Manufacturing Practice (cGMP), excluding infant formulas and meat and poultry products.

The U.S. FDA has raised no questions on the use of other high-intensity sweeteners, including other steviol glycoside preparations, as general purpose sweetening agents that have no restrictions on their specific food uses and use-levels. In addition, the use-levels of high-intensity sweeteners are restricted based on the technological properties of the sweetening agent (*i.e.*, sweetness potency). As a result, considering that

the sweetness profile of high-purity rebaudioside M ($\geq 85\%$ rebaudioside M) is comparable to the sweetness profiles of other high-intensity sweeteners, including other steviol glycoside preparations, the food uses and use-levels of high-purity rebaudioside M ($\geq 85\%$ rebaudioside M) are likely to reflect those currently permitted for other high-intensity sweeteners in the U.S.

1.4 Basis for GRAS

Pursuant to Title 21, Section 170.30 of the Code of Federal Regulations (CFR), high-purity rebaudioside M ($\geq 85\%$ rebaudioside M) manufactured by Tate & Lyle has been concluded to have GRAS status, on the basis of scientific procedures. The GRAS determination is based on information generally available in the public domain pertaining to the safety of steviol glycosides and the enzyme production strains, as discussed herein, and on consensus among a panel of experts who are qualified by scientific training and experience to evaluate the safety of high-purity rebaudioside M ($\geq 85\%$ rebaudioside M) as a general purpose sweetener [see Appendix A, titled “**Expert Panel Report Concerning the Generally Recognized as Safe (GRAS) Status of High-Purity Rebaudioside M ($\geq 85\%$ Rebaudioside M) for Use as a General Purpose Sweetener**”].

1.5 Availability of Information

The data and information that serve as the basis for this GRAS Notification will be made available to the U.S. FDA for review and copying upon request during business hours at the offices of:

Tate & Lyle
5450 Prairie Stone Parkway
Hoffman Estates, IL
USA, 60192

In addition, Tate & Lyle will supply additional data and information should the FDA have any questions regarding this notification during or after the Agency’s review of the notice.

1.6 Freedom of Information Act, 5 U.S.C. 552

It is Tate & Lyle’s view that all data and information presented in Parts 2 through 7 of this notice do not contain any trade secret, commercial, or financial information that is privileged or confidential, and therefore all data and information presented herein are not exempt from the Freedom of Information Act, 5 U.S.C. 552.

Part 2. §170.230 Identity, Method of Manufacture, Specifications, and Physical or Technical Effect

2.1 Identity

2.1.1 Common or Usual Name

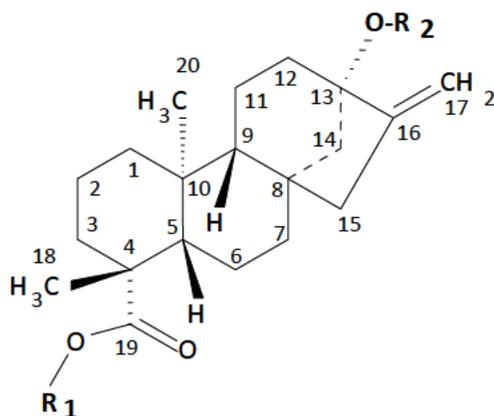
Rebaudioside M; Reb M; Steviol glycosides; TASTEVA® M

2.1.2 Chemical and Physical Characteristics

Tate & Lyle's high-purity rebaudioside M ($\geq 85\%$ rebaudioside M; Reb M) is produced by enzymatic conversion of steviol glycosides ($\geq 95\%$ steviol glycosides) extracted from the leaves of *Stevia rebaudiana* Bertoni using enzymes (2 glucosyltransferases and a sucrose synthase) derived from genetically modified *Escherichia coli* strains derived from *E. coli* K-12. The high-purity rebaudioside M ($\geq 85\%$ rebaudioside M) is a white to off-white powder that has a clean taste with no abnormal or off odor and is freely soluble in water. High-purity rebaudioside M ($\geq 85\%$ rebaudioside M) is approximately 208 times sweeter than sucrose, which is consistent with the sweetness profile of steviol glycosides (FAO, 2016).

The backbone structure for steviol glycosides is shown in Figure 2.1.2-1. All steviol glycosides share a common steviol backbone and differ only with respect to the type and number of glycoside units (*i.e.*, glucose, xylose, rhamnose, fructose, deoxyglucose, galactose, and/or arabinose) conjugated at positions R₁ and R₂. Due to the common steviol backbone, all steviol glycosides share a similar metabolic fate.

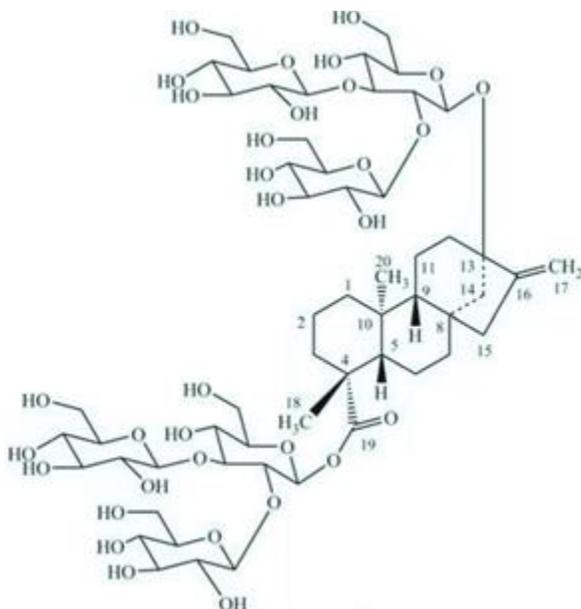
Figure 2.1.2-1 Chemical Structure of Steviol Glycosides



R₁ and R₂ may be a single or multiple glycoside unit, including glucose, xylose, rhamnose, fructose, deoxyglucose, galactose, and/or arabinose.

Rebaudioside M contains 3 glucose units each at R₁ and R₂ (Figure 2.1.2-2). It should be noted that Tate & Lyle's high-purity rebaudioside M ($\geq 85\%$ rebaudioside M) is a highly purified product that contains $\geq 85\%$ Reb M and $\geq 95\%$ total steviol glycosides, which is consistent with the purity criteria for steviol glycosides as established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (JECFA, 2017a). The remaining 5% of the ingredient may also include other steviol glycosides as defined by JECFA.

Figure 2.1.2-2 Chemical Structure for Rebaudioside M



2.2 Method of Manufacturing

Tate & Lyle's high-purity rebaudioside M ($\geq 85\%$ rebaudioside M) is produced by enzymatic conversion of steviol glycosides ($\geq 95\%$ total steviol glycosides) using 2 glucosyltransferases and a sucrose synthase derived from genetically modified *E. coli* strains derived from *E. coli* K-12. These enzymes serve to convert steviol glycosides to Reb M. High-purity rebaudioside M ($\geq 85\%$ rebaudioside M) is manufactured in different stages. In the first stage, the starting material, a steviol glycoside mixture containing $\geq 95\%$ total steviol glycosides, is prepared from the leaves of *S. rebaudiana* Bertonii in accordance with the methodology outlined in the Chemical and Technical Assessment (CTA) for steviol glycosides (FAO, 2016). In the second step, the production strains undergo a fermentation process to produce the 2 glucosyltransferases and sucrose synthase required for the enzymatic conversion reaction. Next, the steviol glycoside mixture and the enzymes are mixed to initiate the conversion of steviol glycosides to Reb M. The resulting steviol glycoside mixture is purified through a series of filtration and washing steps to yield a final product containing $\geq 95\%$ total steviol glycosides and $\geq 85\%$ Reb M. The manufacturing steps are described in more detail in the sections that follow. A schematic overview of the manufacturing process for high-purity rebaudioside M ($\geq 85\%$ rebaudioside M) produced by enzymatic conversion of steviol glycosides is provided in Figure 2.2.3-1 below.

2.2.1 Raw Materials and Processing Aids

All raw materials, processing aids, and purification equipment used to manufacture high-purity rebaudioside M ($\geq 85\%$ rebaudioside M) produced by enzymatic conversion of steviol glycosides are food-grade ingredients¹, permitted by U.S. regulation, have GRAS status, or have been self-affirmed as safe for use in food for their respective uses.

¹ Compliant with the specifications set forth in the Food Chemicals Codex or equivalent international food or pharmacopeia standard (e.g., JECFA, CODEX, United States Pharmacopeia, European Pharmacopoeia).

2.2.2 Enzymes

The 2 glucosyltransferases and sucrose synthase enzymes used in the enzymatic conversion process are derived from genetically modified strains of *E. coli* that are a derivative of *E. coli* K-12, carrying the corresponding synthetic genes to produce the enzymes by fermentation followed by downstream processing. The enzymes are manufactured in an ISO 9001-certified facility and in accordance with cGMP. The parental organism is non-pathogenic and non-toxicogenic and is a Biosafety Level 1 organism according to the National Institute of Health (NIH) (NIH, 2016). Appropriate food-grade specifications have been established for each enzyme which are consistent with the purity specifications for enzyme preparations established by JECFA and the Food Chemical Codex (JECFA, 2006; FCC, 2016). The manufacturing process includes steps in which the enzymes are removed from the final product through filtration and purification processes. Tate & Lyle analyzed 3 non-consecutive batches of high-purity rebaudioside M ($\geq 85\%$ rebaudioside M) for residual protein, which demonstrates successful removal of protein from the final product (see Section 2.3.5 for further details).

2.2.3 Manufacturing Process

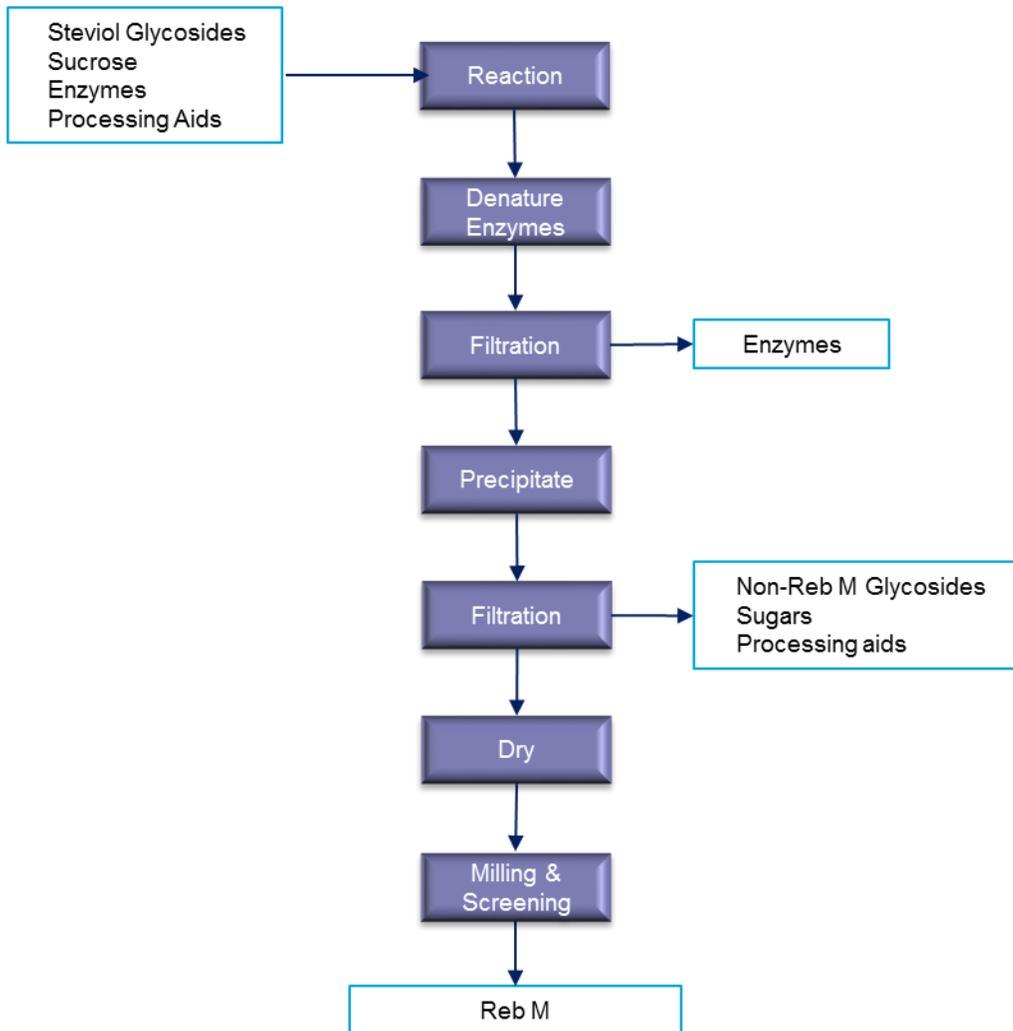
In the first step, a steviol glycoside mixture containing $\geq 95\%$ total steviol glycosides to be used in the enzymatic conversion reaction is produced from *S. rebaudiana* leaves. The manufacturing process is described in detail in GRAS Notice (GRN) 275 (U.S. FDA, 2008). In brief, steviol glycosides are extracted from the stevia leaf by a series of crushing, dissolution, solvent extraction, and precipitation steps that are consistent with the methodology outlined in the CTA for steviol glycosides (FAO, 2016). The steviol glycoside mixture contains $\geq 95\%$ total steviol glycosides, thereby meeting the JECFA specifications for steviol glycosides.

The production strains carrying the expression vector for each corresponding enzyme undergo fermentation and downstream processing to generate the enzymes required for the enzymatic conversion step. Parameters routinely monitored during the fermentation are pH, aeration, agitation, temperature, dissolved oxygen, and microbial growth. Microbiological analysis is also performed on the culture to ensure the absence of contaminants. If evidence of contamination is detected, the fermentation broth is not further processed to recover the enzymes and is discarded. At the end of the fermentation step, triethanolamine is added to the fermentation broth and the cells are mechanically disrupted to release the enzymes into the medium. The solution is then heat-treated to coagulate the host cell proteins and the flocculant is added to aid the clarification. The solution is centrifuged to isolate the solids, which are then removed, and the clarified supernatant is concentrated by ultrafiltration and passed through a 0.2 micron filter to remove any remaining microbial cells and solids. The filtered solution is immediately freeze dried to stabilize the enzyme preparation.

Next, the starting material containing $\geq 95\%$ total steviol glycosides, sucrose, and the reaction processing aids are dissolved in water in the bioconversion reactor. The glucosyltransferases and sucrose synthase are added to the reactor to initiate the enzymatic conversion reaction. The reaction is allowed to proceed until the conversion of the steviol glycosides to Reb M is complete.

The enzyme bioconversion product described above is subjected to conditions which denature the enzymes, resulting in their precipitation out of solution. The precipitate containing the enzymes is removed by filtration. The filtrate is processed to precipitate the rebaudioside M, which are then filtered and treated to remove impurities. Finally, the rebaudioside M product is dried and processed to the final high-purity rebaudioside M product containing $\geq 85\%$ rebaudioside M and $\geq 95\%$ total steviol glycosides.

Figure 2.2.3-1 Schematic Overview of the Manufacturing Process of High-Purity Rebaudioside M (≥85% Rebaudioside M)



2.2.4 Construction of the Production Strains

The production strains are derived from *E. coli* K-12, a non-pathogenic and non-toxic organism belonging to Biosafety Level 1 according to the NIH (NIH, 2016). The recipient strains were modified to confer resistance to phage contamination.

The synthetic genes encoding for each of the 3 enzymes (2 glucosyltransferases and sucrose synthase) were generated based on the native sequence obtained from each respective source organism (which were species of plants or bacteria). The synthetic genes were optimized for expression in *E. coli* and were further modified *via* amino acid mutations to improve thermostability, enzyme solubility, and/or nucleotide

diphosphate utilization. The gene sequences were then inserted into the vector, pCK900 (also known as pCK110900). The expression plasmids carrying each synthetic gene sequence were generated by standard recombinant DNA technology. The plasmid does not contain any DNA cloned from the source organism, and therefore do not contain extraneous unidentified DNA that can be transferred from the donor organism to the production strain.

The expression plasmids do not have any mobility or conjugative sequences, and therefore, it is unlikely that the antibiotic resistance gene² (encoding for chloramphenicol resistance) will be introduced to other bacteria or the environment. The plasmids have been fully sequenced and shown to not carry any sequences of concern.

The production strains are generated by electroporation of the recipient strain with each respective expression plasmid containing the genes encoding for glucosyltransferase or sucrose synthase.

2.3 Product Specifications and Batch Analyses

2.3.1 Product Specifications

Appropriate food-grade specifications have been established for high-purity rebaudioside M ($\geq 85\%$ rebaudioside M) based on the specifications for steviol glycosides established by JECFA (2017a) (Table 2.3.1-1). All analytical methods used to measure each specification parameter are internationally-recognized methods (*e.g.*, United States Pharmacopeia [USP], Association of Official Analytical Chemists [AOAC], or JECFA). Total steviol glycoside content is measured using the high-performance liquid chromatography (HPLC) method described in the JECFA specification monograph for steviol glycosides from *S. rebaudiana* Bertoni (JECFA, 2017a,b).

Table 2.3.1-1 Product Specifications for High-Purity Rebaudioside M ($\geq 85\%$ Rebaudioside M)

Specification Parameter	High-Purity Rebaudioside M ($\geq 85\%$ Rebaudioside M)	Current JECFA Specifications for Steviol Glycosides (JECFA, 2017a)	Method of Analysis
Physical and Chemical Parameters			
Appearance	White to off-white powder	White to light yellow powder	
Total steviol glycosides (anhydrous basis)	$\geq 95\%$	$\geq 95\%$ total steviol glycosides ^a	TN34236 [Monograph 19 (82 nd JECFA Meeting 2016)]
Rebaudioside M	$\geq 85\%$	N/A	TN34240 [Monograph 19 (82 nd JECFA Meeting 2016)]
Loss on drying	$\leq 6\%$	$\leq 6\%$ (105°C, 2h)	TN46040 (CRA E-46)
pH (1% solution)	4.5 to 7.0	4.5 to 7.0	TN60730 (AOAC 981.12)
Residual ethanol	$\leq 5,000$ ppm ($\leq 0.5\%$)	$\leq 0.5\%$	TN64080 (USP 32-NF 27)
Residual methanol	≤ 200 ppm ($\leq 0.02\%$)	$\leq 0.02\%$	TN64080 (USP 32-NF 27)
Total ash	$\leq 1\%$	$\leq 1\%$	TN09560 (AOAC 900.02)
Lead	≤ 1 ppm	≤ 1 ppm	TN44290 (AOAC 993.14)
Arsenic	≤ 1 ppm	≤ 1 ppm	TN44292 (AOAC 993.14)
Cadmium	≤ 1 ppm	NS	TN44291 (AOAC 993.14)
Mercury	≤ 1 ppm	NS	TN44293 (AOAC 993.14)

Microbiological Parameters

² The chloramphenicol acetyltransferase gene used in pCK900 is originally from *E. coli* Tn9 and is already naturally found in many wild-type host cells.

Table 2.3.1-1 Product Specifications for High-Purity Rebaudioside M (≥85% Rebaudioside M)

Specification Parameter	High-Purity Rebaudioside M (≥85% Rebaudioside M)	Current JECFA Specifications for Steviol Glycosides (JECFA, 2017a)	Method of Analysis
Total plate count	<1,000 CFU/g	<1,000 CFU/g	TN10560 (CRA Microbiological Methods I-A)
Mold	<100 CFU/g	<200 CFU/g	TN47010 (CRA Microbiological Methods II-A-1)
Yeast	<100 CFU/g	<200 CFU/g	TN97010 (CRA Microbiological Methods I-A)
Coliforms	<3 MPN/g	NS	TN10510 (CRA Microbiological Methods IV-B)
<i>Escherichia coli</i>	Not detected	Not detected	TN10512 (CRA Microbiological Methods IV-B)
<i>Salmonella</i>	Negative/25 g	Not detected	TN10547 (CRA Microbiological Methods V-A)

AOAC = Association of Official Analytical Chemists; CFU = colony-forming units; CRA = Corn Refiners Association; JECFA = Joint FAO/WHO Expert Committee on Food Additives; MPN = most probable number; N/A = not applicable; NS = not specified; ppm = parts per million; USP = United States Pharmacopeia.

^a Where steviol glycosides “consists of a mixture of compounds containing a steviol backbone conjugated to any number or combination of the principal sugar moieties in any of the orientations occurring in the leaves of *Stevia rebaudiana* Bertoni including, glucose, rhamnose, xylose, fructose, deoxyglucose, galactose, and arabinose”. (JECFA, 2017a).

2.3.2 Batch Analyses

Analysis of 3 non-consecutive lots of high-purity rebaudioside M (≥85% rebaudioside M) produced by enzymatic conversion of steviol glycosides demonstrates that the manufacturing process produces a consistent product that meets the established product specifications. A summary of the batch analyses is presented in Table 2.3.2-1.

Table 2.3.2-1 Summary of the Product Analysis for 3 Non-Consecutive Lots of High-Purity Rebaudioside M (≥85% Rebaudioside M)

Specification Parameter	Limit	Manufacturing Lot No.		
		450237	450618	450931
Appearance	White to off-white powder	Pass	Pass	Pass
Total steviol glycosides (anhydrous basis)	≥95%	98.2	98.5	98.2
Rebaudioside M	≥85%	97.0	97.4	97.2
Loss on drying	≤6%	4.6	3.4	3.4
pH (1% solution)	4.5 to 7.0	Pass	Pass	Pass
Residual ethanol	≤5,000 ppm	<20 ppm	<20 ppm	<20 ppm
Residual methanol	≤200 ppm	<20 ppm	<20 ppm	<20 ppm
Total ash	≤1%	<1%	<1%	<1%
Lead	≤1 ppm	<1 ppm	<1 ppm	<1 ppm
Arsenic	≤1 ppm	<1 ppm	<1 ppm	<1 ppm
Cadmium	≤1 ppm	<1 ppm	<1 ppm	<1 ppm
Mercury	≤1 ppm	<1 ppm	<1 ppm	<1 ppm
Total plate count	<1,000 CFU/g	<1,000 CFU/g	<1,000 CFU/g	<1,000 CFU/g

Table 2.3.2-1 Summary of the Product Analysis for 3 Non-Consecutive Lots of High-Purity Rebaudioside M (≥85% Rebaudioside M)

Specification Parameter	Limit	Manufacturing Lot No.		
		450237	450618	450931
Mold	<100 CFU/g	<10 CFU/g	<10 CFU/g	<10 CFU/g
Yeast	<100 CFU/g	<10 CFU/g	<10 CFU/g	<10 CFU/g
Coliforms	<3 MPN/g	<3 MPN/g	<3 MPN/g	<3 MPN/g
<i>Escherichia coli</i>	Not detected	Not detected	Not detected	Not detected
<i>Salmonella</i>	Negative/25 g	Negative	Negative	Negative

CFU = colony-forming units; MPN = most probable number; NA = not applicable; ppm = parts per million.

2.3.3 Residual Protein Analysis

To provide an indication of the removal of residual protein in the final product, 3 non-consecutive batches of the high-purity rebaudioside M (≥85% rebaudioside M) produced by enzymatic conversion of steviol glycosides (Lot No. 450237, 450618, 450931) were analyzed using the bicinchoninic acid (BCA) assay. The limit of detection was 5 ppm. The results of the analysis were below the detection limit providing further evidence that downstream processing successfully removed the enzymes and other residual proteins from the final product.

2.3.4 Pesticide Residue Analysis

Pesticide residue analysis on the starting material (≥95% total steviol glycosides; Lot No. 419240) demonstrated the absence of residues of commonly used pesticides in the final product.

2.4 Stability Data

A number of scientific and authoritative bodies, including JECFA, the European Food Safety Authority (EFSA), and Food Standards Australia/New Zealand (FSANZ), have reviewed the stability of steviol glycosides. The stability of steviol glycosides also are discussed in several published studies (Chang and Cook, 1983; Kroyer, 1999; Oehme *et al.*, 2017). At their 68th meeting, JECFA evaluated the stability of steviol glycosides under conditions mimicking their use in foods and noted that steviol glycosides do not undergo browning or caramelization when heated and are stable under elevated temperatures (JECFA, 2007). In addition, steviol glycosides (approximately 90 to 94% purity) are stable for at least 180 days when stored at temperatures up to 24°C and pH 2.0 to 4.0. However, at elevated temperatures (80°C), steviol glycoside solutions maintained in water and pH 4.0 and 3.0 for 8 hours showed 4 and 8% decomposition, respectively. At temperatures of 100°C, higher rates of decomposition were observed, with 10 and 40% decomposed at pH 4.0 and 3.0, respectively. These results indicate that the stability of steviol glycosides is pH- and temperature-dependent. Based on the available evidence, JECFA concluded that steviol glycosides are thermally and hydrolytically stable for use in foods and acidic beverages under normal processing and storage conditions.

The U.S. FDA has reviewed the stability of high-purity rebaudioside M preparations in previous GRAS notices (GRN 667 and GRN 512) (U.S. FDA, 2014, 2016). There exists a number of studies on the stability of steviol glycosides, including stevioside, rebaudioside A, and rebaudioside M, under different storage conditions (*e.g.*, in different forms, such as powder and solution, in acidic conditions, and various temperatures) in the publicly-available scientific literature (Wood *et al.*, 1955; Chang and Cook, 1983; Kinghorn, 2002; Cargill, 2008; Merisant, 2008; Chaturvedula *et al.*, 2013; Prakash *et al.*, 2014). These studies are discussed in detail

in GRN 512 and GRN 667 and are incorporated by reference in this notice. Ultimately, the results of these stability studies suggest that the stability of steviol glycosides is pH- and temperature-dependent, which are consistent with the conclusions of JECFA (2007). More recently, a study evaluating the structural stability of 3 commercial batches each of the dried stevia leaves, the first aqueous infusion of the ground stevia, and a high-purity stevia leaf extract ($\geq 95\%$ steviol glycosides) confirmed that the processing steps does not chemically alter or modify the steviol glycoside content (Oehme *et al.*, 2017).

In addition to the stability studies within the scientific literature, storage stability studies on rebaudioside M were discussed in GRN 512 and GRN 667. GRN 512 presented the results of a stability test on 1 batch of Rebpure™ RM95, which contains $\geq 95\%$ rebaudioside M (U.S. FDA, 2014). In this study, a sample of Rebpure™ RM95 was stored at $25 \pm 5^\circ\text{C}$ and relative humidity of $60 \pm 5\%$ for up to 8 weeks. The results of the study demonstrate that the rebaudioside M and the total steviol glycoside content remained $\geq 95\%$ over the course of the 8-week study period. GRN 667 presented the results of an accelerated storage stability study on rebaudioside M ($\geq 95\%$ rebaudioside M) when stored at $40 \pm 2^\circ\text{C}$ and relative humidity of $75 \pm 5\%$ for up to 6 months (U.S. FDA, 2016). Over the course of the accelerated stability study, rebaudioside M was observed to be stable in that the rebaudioside M content did not change over the 6-month period and remained $\geq 95\%$ rebaudioside M.

The results of these storage stability studies are consistent with the results of JECFA (2007) in that the stability of steviol glycosides, including rebaudioside M, are thermally stable under normal storage conditions. Furthermore, while the rebaudioside M content of Tate & Lyle’s high-purity rebaudioside M and that of the preparations described in GRN 512 and GRN 667 are different, (*i.e.*, $\geq 85\%$ vs. $\geq 95\%$, respectively), the total steviol glycosides content are similar in all rebaudioside M preparations (*i.e.*, $\geq 95\%$ total steviol glycosides). Rebaudioside M is expected to exhibit similar chemical stability to other closely related steviol glycosides (*e.g.*, stevioside and rebaudioside A) based on their chemical structure similarity. Therefore, it is anticipated that the results of the stability studies on the rebaudioside M preparations described in GRN 512 and GRN 667, and the results of the stability studies available in the publicly-available scientific literature, can be extended to support the stability of Tate & Lyle’s high-purity rebaudioside M ($\geq 85\%$ rebaudioside M).

Tate & Lyle is currently conducting an accelerated stability study on 1 batch of high-purity rebaudioside M ($\geq 85\%$ rebaudioside M) produced by enzymatic conversion of steviol glycosides (Lot No. 444378). In this study, sample of approximately 50 g of high-purity rebaudioside M ($\geq 85\%$ rebaudioside M) will be stored at 50°C in polyethylene bags for up to 6 months. To mimic the commercial packaging each polyethylene bag was double heat-sealed and placed in a secondary bag, which was also double heat-sealed. Total steviol glycosides and Reb M content were measured by HPLC at baseline and at 1, 3, and 6 months. The moisture content was measured by Karl Fischer analysis. Preliminary results at 3 months indicate no significant changes in Reb M or steviol glycosides content (Table 2.4-1). The accelerated stability study is currently on-going.

Table 2.4-1 Results of an Accelerated Stability Study on 1 Batch of High-Purity Rebaudioside M ($\geq 85\%$ Rebaudioside M) (Lot No. 444378) (Study Currently On-going)

Parameter	Month			
	0 (baseline)	1	3	6
Total steviol glycosides (%) (dry basis)	93.4	94.9	95.8	TBD
Rebaudioside M (%) (dry basis)	90.0	91.3	92.1	TBD

TBD = to be determined.

Part 3. §170.235 Dietary Exposure

3.1 Intended Use of High-Purity Rebaudioside M (≥85% Rebaudioside M) and Levels of Use in Foods

High-purity rebaudioside M (≥85% rebaudioside M) is intended for use as a general purpose sweetener in accordance with cGMP, excluding infant formulas and meat and poultry products. High-purity rebaudioside M (≥85% rebaudioside M) has a sweetness intensity of approximately 208 times that of sucrose. To date, the U.S. FDA raised no questions to the use of other high-intensity sweeteners, including other steviol glycoside preparations, as general purpose sweeteners that have no restrictions on their specific food uses and use-levels. In addition, the use-levels of high-intensity sweeteners are restricted based on the technological properties of the sweetening agent (*i.e.*, sweetness potency). Therefore, considering that steviol glycosides, including the ingredient that is the subject of this GRAS notice, are characterized by a sweetness profile that is, for the most part, comparable to other high-intensity sweeteners, the uses and use-levels of high-purity rebaudioside M (≥85% rebaudioside M) produced by enzymatic conversion of steviol glycosides will likely reflect those currently permitted for other high-intensity sweeteners in the U.S.

3.2 Estimated Consumption of High-Purity Rebaudioside M (≥85% Rebaudioside M) Based Upon Intended Food Uses

3.2.1 History of Consumption of Steviol Glycosides

Stevia rebaudiana and the individual steviol glycosides derived from the plant have been consumed as sweeteners in various foods and beverages since the late 1800s (Geuns, 2003). According to Blumenthal (1995) and Geuns (2003), the native peoples of Brazil and Paraguay have consumed the *S. rebaudiana* plant for hundreds of years as a food ingredient and as a tea. Similarly, *S. rebaudiana* became a popular herbal tea ingredient in the U.S. in the 1980s (Blumenthal, 1995; Ferlow, 2005). Stevioside, the first isolated steviol glycoside from the *S. rebaudiana* leaf, has been consumed in Japan for more than 30 years (Geuns, 2003; Ferlow, 2005). Approximately 160,000 metric tons of stevioside, as sucrose equivalents, were reportedly consumed in Asia in 1995; in 1999, this level increased to approximately 200,000 metric tons as sucrose equivalents (International Sugar Organization, 2001).

3.2.2 Estimated Consumption of High-Purity Rebaudioside M (≥85% Rebaudioside M) from Proposed Food Uses

The dietary consumption of various steviol glycoside preparations have been estimated using a post-market surveillance approach as outlined in a number of GRAS notices for steviol glycosides submitted to the U.S. FDA (*e.g.*, GRN 667, 715, and 733). Generally, this approach uses the data from Renwick (2008) in which dietary exposure to Reb A was estimated based on the available post-market surveillance data for other high-intensity sweeteners, and by assuming full replacement of the currently approved high-intensity sweeteners with the new sweetener [*i.e.*, high-purity rebaudioside M (≥85% rebaudioside M)]. While conservative, this approach yields intake estimates that are realistic as they reflect actual post-market intakes of high-intensity sweeteners. Renwick (2008) estimated the average and high-end dietary intakes of Reb A as sucrose equivalents in various population groups, such as non-diabetic and diabetic adults and children, and adjusted the values accordingly using the sweetness intensity of Reb A relative to sucrose (approximately 200).

This post-market surveillance approach can be used to estimate the dietary intakes of high-purity rebaudioside M ($\geq 85\%$ rebaudioside M) (Table 3.2.2-1). Tate & Lyle determined that high-purity rebaudioside M ($\geq 85\%$ rebaudioside M) is approximately 208 times sweeter than sucrose based on the results of a sweetness potency test. The estimated intake values for high-purity rebaudioside M ($\geq 85\%$ rebaudioside M) were calculated based on the sweetness potency and the molecular weight of Reb M.

Table 3.2.2-1 Estimated Consumption High-Purity Rebaudioside M ($\geq 85\%$ Rebaudioside M) Using the Intense Sweetener Intake Assessment Methodology described by Renwick (2008)

Population Group	Intakes of Intense Sweeteners (expressed as sucrose equivalents) (mg/kg bw/day)		Consumption Estimates			
	Average Consumer	High Consumer	High-Purity Rebaudioside M ($\geq 85\%$ Rebaudioside M) ^a (mg/kg bw/day)		High-Purity Rebaudioside M ($\geq 85\%$ Rebaudioside M) as steviol equivalents ^{a,b} (mg/kg bw/day)	
			Average Consumer	High Consumer	Average Consumer	High Consumer
Non-diabetic adults	255	675	1.23	3.25	0.30	0.80
Diabetic adults	280	897	1.35	4.31	0.33	1.06
Non-diabetic children	425	990	2.04	4.76	0.50	1.17
Diabetic children	672	908	3.23	4.37	0.79	1.07

bw = body weight.

^a Approximately 208 times as sweet as sucrose.

^b Calculated based on the molecular weights of steviol (318.45 g/mol) and Reb M (1,291.3 g/mol) [steviol conversion factor of 0.25].

For non-diabetic adults, average and high-end intakes of high-purity rebaudioside M ($\geq 85\%$ rebaudioside M) of up to 0.30 and 0.80 mg/kg body weight/day expressed as steviol equivalents, respectively, were calculated. For diabetic adults, average and high-end intakes were slightly higher at up to 0.33 and 1.06 mg/kg body weight/day. Average and high-end exposures to high-purity rebaudioside M ($\geq 85\%$ rebaudioside M), expressed as steviol equivalents, in non-diabetic children were calculated to be up to 0.50 and 1.17 mg/kg body weight/day, respectively. Although average intakes of high-purity rebaudioside M ($\geq 85\%$ rebaudioside M), expressed as steviol equivalents, were estimated to be higher at up to 0.79 mg/kg body weight/day in diabetic children compared to values for non-diabetic children, high-end values in diabetic children (1.07 mg/kg body weight/day) were lower than high-end values in non-diabetic children. The predicted intakes of high-purity rebaudioside M ($\geq 85\%$ rebaudioside M), expressed as steviol equivalents, are all below the current acceptable daily intake (ADI) defined by the JECFA for steviol glycosides (FAO, 2016) of 0 to 4 mg/kg body weight/day as steviol.

In 2008, JECFA considered various intake models for the estimation of dietary exposure to steviol glycosides, including the intake analysis conducted by Renwick (2008) as part of their evaluation of the safety of steviol glycosides. Although higher intake estimates than those presented by Renwick (2008) were identified using other methodologies, including ones considering replacement of all sweeteners used in or as food (up to approximately 6 mg/kg body weight/day, expressed as steviol equivalents), JECFA noted that such replacement estimates were highly conservative and that actual exposures to steviol glycosides (expressed as steviol equivalents) would be 20 to 30% of these values (1 to 2 mg/kg body weight/day, expressed as steviol equivalents). JECFA also noted that the post-market surveillance approach further confirmed the lower intake estimate range.

Part 4. §170.240 Self-Limiting Levels of Use

The use of high-purity rebaudioside M ($\geq 85\%$ rebaudioside M) is largely limited by the desired sweetness intended for a particular food or beverage product. Therefore, the use of high-purity rebaudioside M ($\geq 85\%$ rebaudioside M) as a general purpose sweetener in foods is self-limiting based on its organoleptic properties.

Part 5. §170.245 Experience Based on Common Use in Food Before 1958

Not applicable as high-purity rebaudioside M ($\geq 85\%$ rebaudioside M) was not used in food before 1958.

Part 6. §170.250 Narrative and Safety Information

The safety of steviol glycosides has been extensively reviewed by the U.S. FDA in a number of GRAS notices. The Agency raised no objections to over 50 GRAS notices describing the GRAS status of major individual steviol glycosides, including stevioside, rebaudiosides A, C, D, and X/M, mixtures of steviol glycosides, and glucosylated and enzyme-modified steviol glycosides (GRNs 252, 253, 275, 278, 282, 287, 303, 304, 318, 323, 329, 337, 348, 349, 354, 365, 367, 369, 375, 380, 388, 389, 393, 395, 418, 448, 452, 456, 461, 467, 473, 493, 512, 516, 536, 548, 555, 607, 619, 626, 632, 638, 656, 662, 667, 702, 715, 733). In addition to the U.S. FDA, the safety of steviol glycosides has been reviewed by several scientific bodies and regulatory agencies, including JECFA, European Commission's Scientific Committee on Food (SCF), EFSA, FSANZ, and Health Canada. The existing safety database on steviol glycosides includes an extensive evaluation of the metabolism and pharmacokinetics of steviol glycosides in rodents and humans, and a standard battery of toxicological tests, including acute toxicity, short- and long-term toxicity and carcinogenicity, reproductive and developmental toxicity, *in vitro* and *in vivo* mutagenicity and genotoxicity, as well as several human studies.

Much of the early studies investigating the safety of steviol glycosides were conducted with stevioside, the predominant steviol glycoside in *S. rebaudiana* leaves (Aze *et al.*, 1991; Toyoda *et al.*, 1997). Since then, additional toxicity testing has been conducted on rebaudioside A and D (Curry and Roberts, 2008; Curry *et al.*, 2008; Nikiforov and Eapen, 2008; Williams and Burdock, 2009). Due to the common metabolic fate of steviol glycosides, the scientific bodies and regulatory agencies described above have extended their safety opinions to include all steviol glycosides, rather than individual steviol glycosides (JECFA, 2017a,b). Thus, considering that the existing safety database on steviol glycosides has been extensively reviewed by the U.S. FDA, the pertinent generally available data and information used to support the safety of steviol glycosides, including major individual steviol glycosides and other steviol glycoside mixtures/preparations, is incorporated by reference to information cited within prior GRAS notifications. Updated searches of the scientific literature were conducted through March 2018 to identify new data and information relevant to the safety of steviol glycosides that have been published since the U.S. FDA's last review³. Given the shared metabolic fate of steviol glycosides, the safety of high-purity rebaudioside M ($\geq 85\%$ rebaudioside M) produced by enzymatic conversion of steviol glycosides can be supported based on the existing safety

³ At the time of preparation of this GRAS notice, GRN 733 was the most recent steviol glycoside GRAS notice to receive a "no questions" letter from the U.S. FDA which summarized literature prior to October 2017.

database for steviol glycosides, the safety conclusions for steviol glycosides by JECFA and other scientific and regulatory authorities/bodies, and the safety of the production strains.

6.1 Absorption, Distribution, Metabolism, and Elimination

An extensive database exists outlining the metabolic fate (absorption, distribution, metabolism, and elimination) of steviol glycosides. The available data and information on the metabolic fate of individual steviol glycosides as discussed in detail in several GRAS notices (*e.g.*, GRN 619, 626, 667) is incorporated by reference in this dossier. Briefly, steviol glycosides are not hydrolyzed in the upper gastrointestinal tract due to the presence of β -glycosidic bonds; the unchanged steviol glycosides enter the colon and are subject to microbial degradation by the gut microflora, resulting in the release of the aglycone steviol (Wingard *et al.*, 1980; Hutapea *et al.*, 1997; Gardana *et al.*, 2003; Koyama *et al.*, 2003a,b; Geuns *et al.*, 2003, 2007; Renwick and Tarka, 2008; Nikiforov *et al.*, 2013; Purkayastha *et al.*, 2016). Steviol glycosides are hydrolyzed sequentially, in which one sugar moiety is removed at a time, with the degradation rates dependent on the structural complexity of each steviol glycoside (Wingard *et al.*, 1980; Koyama *et al.*, 2003b). Despite the differences in chemical structure, however, the rates of hydrolysis of different steviol glycosides to steviol are relatively similar, especially during the first 24 hours of incubation in *in vitro* metabolic studies with human fecal homogenates (Purkayastha *et al.*, 2014, 2015, 2016). Following microbial degradation, the steviol metabolite is absorbed systemically into the portal vein and distributed to the liver, spleen, adrenal glands, fat, and blood (Nakayama *et al.*, 1986; Sung, 2002 [unpublished]; Koyama *et al.*, 2003b; Wang *et al.*, 2004; Roberts and Renwick, 2008). Steviol is conjugated to glucuronic acid to form steviol glucuronide in the liver. The steviol glucuronide metabolite and any unconjugated steviol or unhydrolyzed fraction of the administered glycosides are excreted primarily in the urine, and, to a lesser extent, feces in humans (Wingard *et al.*, 1980; Nakayama *et al.*, 1986; Kraemer and Maurer, 1994; Sung, 2002 [unpublished]; Geuns and Pietta, 2004 [unpublished]; Simonetti *et al.*, 2004; Geuns *et al.*, 2006, 2007; Roberts and Renwick, 2008; Wheeler *et al.*, 2008).

In summary, due to the common molecular structure for steviol glycosides, consisting of a steviol backbone conjugated to different numbers and types of sugar moieties, all individual steviol glycosides share a common metabolic fate, as described above. Therefore, the safety database that has been established for individual steviol glycosides (*e.g.*, stevioside, Reb A, Reb D) can be extrapolated to support the safe use of purified steviol glycosides in general, regardless of the steviol glycoside distribution of the preparation, including high-purity rebaudioside M ($\geq 85\%$ rebaudioside M) produced by enzymatic conversion of steviol glycosides.

6.2 Summary of Safety Evaluations on Steviol Glycosides by Scientific and Regulatory Authorities/Bodies

The safety of steviol glycosides was reviewed by JECFA at their 51st, 63rd, 68th, 69th, and 82nd meetings in 1998, 2004, 2007, 2008, and 2016, respectively. In addition, the safety of steviol glycosides has been reviewed by FSANZ, the European Commission's SCF, the EFSA, and Health Canada (SCF, 1985, 1999; FSANZ, 2008; EFSA, 2010, 2015; Health Canada, 2012). These scientific bodies and regulatory agencies have unanimously concluded that consumption of steviol glycosides is not a safety concern and have established an ADI of 0 to 4 mg/kg body weight, expressed as steviol equivalents. Subsequent to these evaluations, EFSA concluded that "*extending the current specifications to include [two additional steviol glycosides], rebaudiosides D and M, as alternatives to Reb A in the predominant components of steviol glycosides would not be of safety concern*" (EFSA, 2015), while JECFA, FSANZ, and Health Canada recently expanded the definition of steviol glycosides to include all individual steviol glycosides present in the *S. rebaudiana* Bertoni leaf (FSANZ, 2017; Health Canada, 2017; JECFA, 2017a,b). In addition to these safety evaluations,

the U.S. FDA has reviewed the safety of over 50 different steviol glycoside preparations and has consistently raised no objections regarding the GRAS status of steviol glycosides.

In these evaluations, the safety data and information that were reviewed by these scientific bodies and regulatory agencies were generally available in the published scientific literature. In a 2-year study in rats, no carcinogenicity or adverse effects on any study parameter were observed, and a no-observed-adverse-effect level (NOAEL) of 970 mg/kg body weight/day, equivalent to 383 mg/kg body weight/day as steviol, was determined (Toyoda *et al.*, 1997). The results of the study by Toyoda *et al.* (1997) was the basis for the established ADI of 0 to 4 mg/kg body weight, expressed as steviol equivalents, for steviol glycosides following application of a safety factor of 200 (JECFA, 2006; FSANZ, 2008; EFSA, 2010; Health Canada, 2012).

6.3 Additional Safety Data for Steviol Glycosides

The safety of steviol glycosides has been extensively reviewed in a number of GRAS notifications submitted to the U.S. FDA, as outlined above, which are incorporated by reference in this notice. The safety of steviol glycosides was most recently evaluated by the U.S. FDA in its evaluation of GRN 733 for purified steviol glycosides, which included a comprehensive search of the scientific literature to capture publications relevant to the safety of steviol glycosides up to October 2017. In order to identify new data related to the safety of steviol glycosides following the U.S. FDA review of GRN 733, a comprehensive search of the scientific literature was conducted from July 2017 to March 2018. The search was limited to articles with full texts within peer-reviewed scientific journals. The following databases were searched: Adis Clinical Trials Insight, AGRICOLA, AGRIS, Allied & Complementary Medicine™, BIOSIS® Toxicology, BIOSIS Previews®, CAB ABSTRACTS, Embase®, Foodline®: SCIENCE, FSTA®, MEDLINE®, NTIS: National Technical Information Service, and ToxFile®. The studies identified included genotoxicity studies and several studies in animals evaluating the safety, antidiabetic, and immune effects of steviol glycosides. In general, the results of these recent studies provide further support for the safety of steviol glycosides as they do not contradict the safety conclusion on steviol glycosides as established by a number of authoritative scientific bodies (*e.g.*, JECFA, FSANZ, the U.S. FDA, the EFSA, and Health Canada).

6.4 Safety of the Production Strains and the Enzymes

The production strains used to generate the glucosyltransferases and sucrose synthase enzymes are derived from the recipient strain initially derived from *E. coli* K-12. The genome of *E. coli* K-12 has been sequenced and confirms the absence of antibiotic resistance genes and other sequences of concern (Blattner *et al.*, 1997; Hayashi *et al.*, 2006; NCBI, 2018). Furthermore, the *E. coli* parental strain is a member of the well-defined family *Enterobacteriaceae*. The synthetic genes introduced into the parental strain are derived from plant and microorganism sources. The safety of the glucosyltransferases and sucrose synthase enzymes was evaluated using the Pariza and Johnson (2001) decision tree and was determined to be “accepted” on the basis that the final product meets the established JECFA specifications, and that the enzymes are absent in the final product. The manufacturing process includes a step to denature the enzymes, and purification steps to remove the production strains and the enzymes from the final product. Analysis of 3 non-consecutive batches of high-purity rebaudioside M (≥85% rebaudioside M) for residual protein demonstrated that the enzymes and other residual proteins were successfully removed from the final product.

6.4.1 History of Use and the Production Strain

E. coli K-12 has been in use as a laboratory organism for over 50 years and it constitutes one of the most extensively characterized microorganisms (Bachmann, 1972; Jensen, 1993). Along with its use in laboratory

research, *E. coli* K-12 has a long history of safe use in the food and pharmaceutical industries. Chymosin, a food enzyme preparation used in the production of cheese, derived from a genetically modified *E. coli* K-12 strain was affirmed as GRAS by the U.S. FDA in 1990 (Flamm, 1991; Olempska-Beer *et al.*, 2006). In addition, GRN 624 concerning D-allulose 3-epimerase derived from a strain of *E. coli* K-12 received “no questions” from the Agency regarding its GRAS status for use in the production of D-allulose and other keto sugars (U.S. FDA, 2016).

6.4.2 Pathogenicity/Toxicogenicity of the Parental Strain

E. coli K-12 is not considered a human or animal pathogen and has been classified as Biosafety Level 1 according to the NIH Guidelines (NIH, 2016). *E. coli* K-12 is often used as a reference organism when investigating the virulence factors of pathogenic *E. coli* strains as it is non-pathogenic (Blanc-Potard *et al.*, 2002; Kaper *et al.*, 2004). This species and its derivatives are unable to colonize the mammalian gastrointestinal tract, and do not produce toxins such as Shiga toxin, and are unable to persist in the soil and water (Bogosian *et al.*, 1996; U.S. EPA, 1997). As previously described, the parental strain does not carry any introduced antibiotic resistance genes and the complete genome of this strain has been sequenced, confirming the absence of any toxigenic potential (Blattner *et al.*, 1997; Hayashi *et al.*, 2006).

The potential pathogenicity of the enzymes (glucosyltransferases and sucrose synthase) was investigated using prediction software MP3 tools (available at <http://metagenomics.iiserb.ac.in/mp3/algorithm.php>) which uses an Support Vector Machine (SVM), Hidden Markov Model (HMM), or integrated SVM-HMM approach to predict pathogenic proteins in both genomic and metagenomic datasets. Each enzyme was searched using the MP3 tools using the default settings (threshold of -0.2 and a protein sequence length of 30 amino acids). All enzymes were predicted to be non-pathogenic using each SVM, HMM, or integrated SVM-HMM approach.

6.4.3 Potential Allergenicity of the Enzymes

The potential allergenicity of the enzymes (glucosyltransferases and sucrose synthase) was investigated using an *in silico* approach. A sequence homology search was conducted according to the approach outlined by the Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) (2001) and the WHO/FAO (2009) using the AllergenOnline Database Version 18 (available at <http://www.allergenonline.org>; updated January 2018) maintained by the Food Allergy Research and Resource Program (FARRP) of the University of Nebraska (FARRP, 2018). This was done to confirm that the enzymes do not contain amino acid sequences similar to other known allergens that might produce an allergenic response. The database contains a comprehensive list of putative allergenic proteins developed *via* a peer reviewed process for the purpose of evaluating food safety.

No matches were identified from searching with the full amino acid sequence for each enzyme. According to the FARRP guidelines, an identity threshold of greater than 50% or an E-score lower than 1×10^{-7} suggest cross-reactivity with the known allergen to be a possibility.

A second homology search was conducted according to the approach outlined by the FAO/WHO (2001) and the WHO/FAO (2009). In accordance with this guideline, the AllergenOnline database was searched using a sliding window of 80-amino acid sequences (segments 1-80, 2-81, 3-82, *etc.*) derived from the full-length amino acid sequence for each enzyme. The 80-amino acid alignment search was conducted using default settings (*E* value cutoff = 1 and maximum alignments of 20). Using this search strategy, again no matches were identified.

Based on the information provided above, no evidence exists to suggest that the enzymes used in the enzymatic conversion of steviol glycosides to Reb M would be associated with an allergenic response.

6.5 Expert Panel Evaluation

Tate & Lyle has concluded that high-purity rebaudioside M ($\geq 85\%$ rebaudioside M) produced by enzymatic conversion of steviol glycosides, meeting appropriate food-grade specifications and manufactured consistent with cGMP, is GRAS for use as an ingredient in various food products, as described in Part 1.3, on the basis of scientific procedures. Tate & Lyle's high-purity rebaudioside M ($\geq 85\%$ rebaudioside M) produced by enzymatic conversion of steviol glycosides is substantially equivalent to other steviol glycoside products currently on the U.S. market, including those extracted from the leaves of *S. rebaudiana*.

The GRAS status of high-purity rebaudioside M ($\geq 85\%$ rebaudioside M) produced by enzymatic conversion of steviol glycosides is based on conclusions of scientific bodies and regulatory authorities regarding steviol glycoside safety, data generally available in the public domain pertaining to the safety of steviol glycosides, and a unanimous opinion among a panel of experts ('the Expert Panel'), who are qualified by scientific training and experience to evaluate the safety of food ingredients. The Expert Panel consisted of the following qualified scientific experts: Michael W. Pariza, Ph.D. (University of Wisconsin-Madison), I. Glenn Sipes, Ph.D. (University of Arizona), and Stanley M. Tarka Jr., Ph.D. (The Tarka Group Inc., and The Pennsylvania State University, College of Medicine).

The Expert Panel, convened by Tate & Lyle, independently and critically evaluated all data and information presented herein, and concluded that high-purity rebaudioside M ($\geq 85\%$ rebaudioside M) produced by enzymatic conversion of steviol glycosides is GRAS for use as a general purpose sweetener, as described in Section 1.3, based on scientific procedures. A summary of data and information reviewed by the Expert Panel and evaluation of such data as it pertains to the proposed GRAS uses of high-purity rebaudioside M ($\geq 85\%$ rebaudioside M), are presented in Appendix A.

6.6 Conclusions

Based on the data and information presented herein, Tate & Lyle has concluded high-purity rebaudioside M ($\geq 85\%$ rebaudioside M) produced by enzymatic conversion of steviol glycosides, meeting appropriate food-grade specifications, and manufactured according to cGMP, is safe for use as a general purpose sweetener as presented in Section 1.3. Tate & Lyle also has further concluded that pivotal data and information relevant to the safety of high-purity rebaudioside M ($\geq 85\%$ rebaudioside M) produced by enzymatic conversion of steviol glycosides are publicly available and therefore the intended uses of high-purity rebaudioside M ($\geq 85\%$ rebaudioside M) can be concluded to be GRAS on the basis of scientific procedures.

Part 7. §170.255 List of Supporting Data and Information

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