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27 April 2018

Dr. Paulette Gaynor
Office of Food Additive Safety (FHS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Campus Drive
College Park, MD 20740

Re: GRAS Notice for High-Purity Rebaudioside M

Dear Dr. Gaynor

In accordance with 21 CFR §170 Subpart E consisting of §170.203 through 170.285, Tate & Lyle Americas LLC hereby informs the United States Food and Drug Administration of the conclusion that High-Purity Rebaudioside M, manufactured by Tate & Lyle Americas LLC, as defined in the enclosed documents, is GRAS under the specified conditions of use as a food ingredient on the basis of scientific procedures, and therefore, is not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act. Information supporting the GRAS status of the High-Purity Rebaudioside M, which includes detailed information on the notified substance and a summary of the basis of the safety of High-Purity Rebaudioside M, under the intended conditions of use, also are enclosed for review by the Agency.

I hereby certify that the enclosed electronic files were scanned for viruses prior to submission and are thus certified as being virus-free using Symantec Endpoint Protection 12.1.5.

Should you have any questions or concerns regarding this GRAS Notice, please do not hesitate to contact me at any point during the review process so that we may provide a response in a timely manner.

Sincerely,

(b) (6)

Susan M. Potter, PhD
Director, Scientific and Regulatory Affairs
Tate & Lyle Americas LLC
susan.potter@tateandlyle.com

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,

(b) (6)

April 27, 2018

Susan M. Potter, PhD
Director, Scientific and Regulatory Affairs
Tate & Lyle Americas LLC
susan.potter@tateandlyle.com

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 (≥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 (≥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 (≥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 (≥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 (≥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

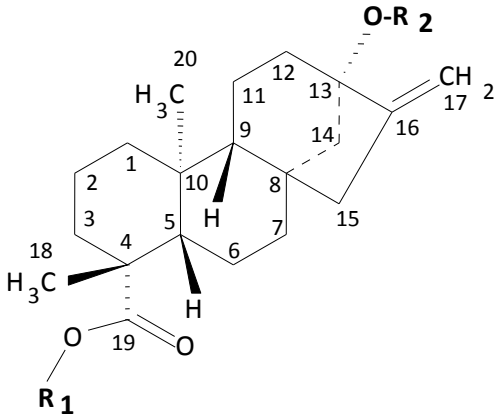
Rebaudioside M; Reb M; Steviol glycosides; TASTEVA® M

2.1.2 Chemical and Physical Characteristics

Tate & Lyle's high-purity rebaudioside M (≥85% rebaudioside M; Reb M) is produced by enzymatic conversion of steviol glycosides (≥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 (≥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 (≥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 R_1 and R_2. Due to the common steviol backbone, all steviol glycosides share a similar metabolic fate.

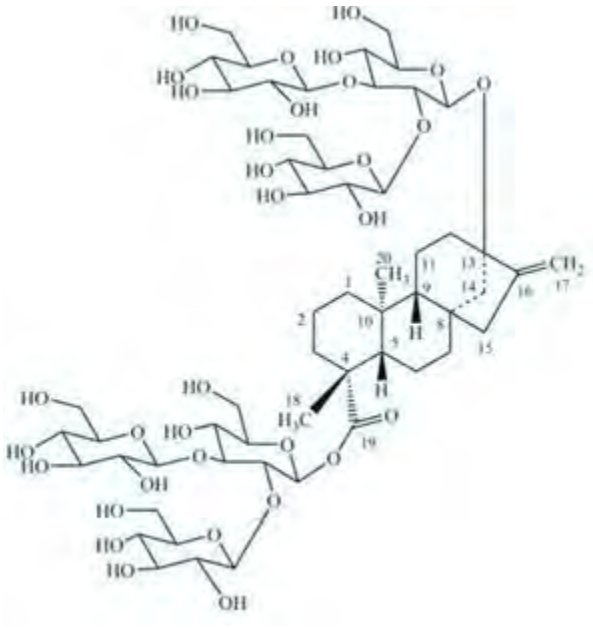
Figure 2.1.2-1 Chemical Structure of Steviol Glycosides



R_1 and R_2 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 R_1 and R_2 (Figure 2.1.2-2). It should be noted that Tate & Lyle's high-purity rebaudioside M (≥85% rebaudioside M) is a highly purified product that contains ≥85% Reb M and ≥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 (≥85% rebaudioside M) is produced by enzymatic conversion of steviol glycosides (≥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 (≥85% rebaudioside M) is manufactured in different stages. In the first stage, the starting material, a steviol glycoside mixture containing ≥95% total steviol glycosides, is prepared from the leaves of *S. rebaudiana* Bertoni 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 ≥95% total steviol glycosides and ≥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 (≥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 (≥85% rebaudioside M) produced by enzymatic conversion of steviol glycosides are food-grade ingredients[1], 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-toxigenic 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 (≥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

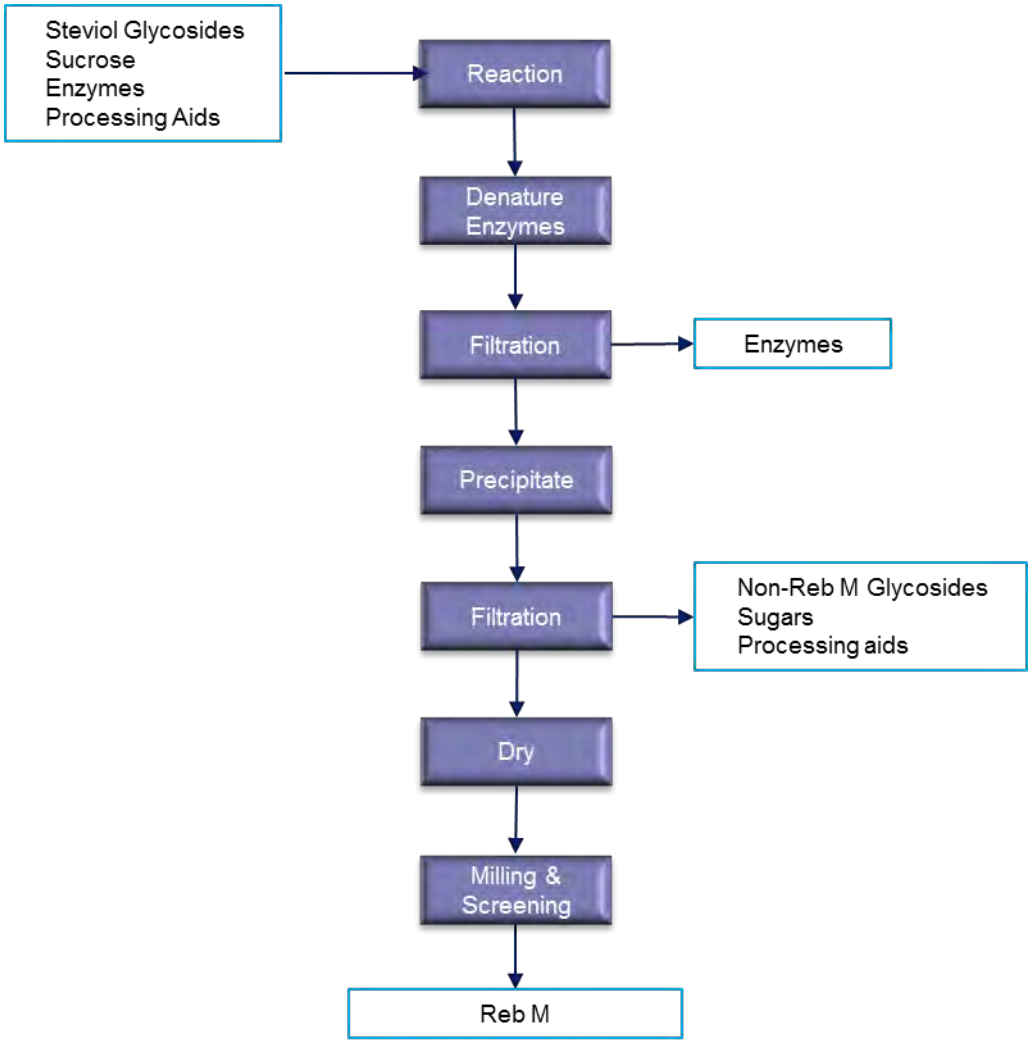
In the first step, a steviol glycoside mixture containing ≥95% total steviol glycosides to be used in the enzymatic conversion reaction is produced from *S. rebaudiana* leaves. The manufacturing process is described in detail in GRAS Notice (GRN) 275 (U.S. FDA, 2008). In brief, steviol glycosides are 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 ≥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 ≥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 ≥85% rebaudioside M and ≥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-toxigenic 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 gene[2] (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 (≥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 (≥85% Rebaudioside M)

Specification Parameter	High-Purity Rebaudioside M (≥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)	≥95%	≥95% total steviol glycosides[a]	TN34236 [Monograph 19 (82nd JECFA Meeting 2016)]
Rebaudioside M	≥85%	N/A	TN34240 [Monograph 19 (82nd JECFA Meeting 2016)]
Loss on drying	≤6%	≤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	≤5,000 ppm (≤0.5%)	≤0.5%	TN64080 (USP 32-NF 27)
Residual methanol	≤200 ppm (≤0.02%)	≤0.02%	TN64080 (USP 32-NF 27)
Total ash	≤1%	≤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)
Escherichia coli	Not detected	Not detected	TN10512 (CRA Microbiological Methods IV-B)
Salmonella	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.		
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
Escherichia coli	Not detected	Not detected	Not detected	Not detected
Salmonella	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 (≥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 ≥95% rebaudioside M (U.S. FDA, 2014). In this study, a sample of Rebpure™ RM95 was stored at 25±5°C and relative humidity of 60±5% for up to 8 weeks. The results of the study demonstrate that the rebaudioside M and the total steviol glycoside content remained ≥95% over the course of the 8-week study period. GRN 667 presented the results of an accelerated storage stability study on rebaudioside M (≥95% rebaudioside M) when stored at 40±2°C and relative humidity of 75±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 ≥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.,* ≥85% *vs.* ≥95%, respectively), the total steviol glycosides content are similar in all rebaudioside M preparations (*i.e.,* ≥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 (≥85% rebaudioside M).

Tate & Lyle is currently conducting an accelerated stability study on 1 batch of high-purity rebaudioside M (≥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 (≥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 (≥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 (≥85% rebaudioside M) (Table 3.2.2-1). Tate & Lyle determined that high-purity rebaudioside M (≥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 (≥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 (≥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			
			High-Purity Rebaudioside M (≥85% Rebaudioside M)[a] (mg/kg bw/day)		High-Purity Rebaudioside M (≥85% Rebaudioside M) as steviol equivalents[a,b] (mg/kg bw/day)	
	Average Consumer	High Consumer	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 (≥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 (≥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 (≥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 (≥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 (≥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 (≥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 (≥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 review[3]. Given the shared metabolic fate of steviol glycosides, the safety of high-purity rebaudioside M (≥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 (≥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 $1x10^{-7}$ 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 (≥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 (≥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 (≥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 (≥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 (≥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 (≥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 (≥85% rebaudioside M) produced by enzymatic conversion of steviol glycosides are publicly available and therefore the intended uses of high-purity rebaudioside M (≥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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Expert Panel Report Concerning the Generally Recognized as Safe (GRAS) Status of High-Purity Rebaudioside M (≥85% Rebaudioside M) for Use as a General Purpose Sweetener

6 April 2018

INTRODUCTION

Tate & Lyle intends to market high-purity rebaudioside M (≥85% rebaudioside M) for use as a general purpose sweetener in the United States (U.S.). Steviol glycosides have historically been obtained by hot water extraction from the leaves of *Stevia rebaudiana* Bertoni and solvent purification. More than 40 different steviol glycosides have been identified in the leaf extracts to date. Tate & Lyle has developed an alternative manufacturing process to produce rebaudioside M. Tate & Lyle's high-purity rebaudioside M (≥85% rebaudioside M) is produced through enzymatic bioconversion of steviol glycosides (≥95% total steviol glycosides) extracted from *S. rebaudiana* Bertoni using glucosyltransferases and sucrose synthase enzymes derived from genetically modified *Escherichia coli* strains derived from *E. coli* K-12. High-purity rebaudioside M is comprised of ≥85% rebaudioside M and ≥95% total steviol glycosides, which meets or exceeds the ≥95% steviol glycoside purity criteria established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the Food Chemicals Codex.

At the request of Tate & Lyle, an Expert Panel of independent scientists, qualified by their relevant national and international experience and scientific training to evaluate the safety of food ingredients, was specially convened to conduct a critical and comprehensive evaluation of the available pertinent data and information, and to determine whether, under the conditions of intended use as a sweetening agent, high-purity rebaudioside M (≥85% rebaudioside M) would be Generally Recognized as Safe (GRAS), based on scientific procedures. The Expert Panel consisted of the below-signed 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). For purposes of the Expert Panel's evaluation, "safe" or "safety" means there is a reasonable certainty in the minds of competent scientists that the substance is not harmful under the intended conditions of use, as defined by the U.S. Food and Drug Administration (FDA) in 21 CFR 170.3(i) (U.S. FDA, 2017).

The Expert Panel independently and collectively evaluated a dossier titled "*Documentation Supporting TASTEVA® M as Generally Recognized as Safe (GRAS) for Use as a General Purpose Sweetener*", which included a comprehensive summary of scientific information on high-purity rebaudioside M (≥85% rebaudioside M). This dossier was prepared with information available in the public domain and also included details pertaining to the manufacturing method, product specifications and supporting batch analyses, intended uses and use-levels in food and beverages, consumption estimates for all intended uses, and a summary of the scientific literature pertaining to the safety of steviol glycosides. The Expert Panel also evaluated other information deemed appropriate or necessary.

Following their independent and critical evaluation of such data and information, the Expert Panel convened on 6 April 2018 *via* teleconference and unanimously concluded that the intended use described herein for high-purity rebaudioside M (≥85% rebaudioside M), meeting appropriate food-grade specifications as described in the supporting dossier and manufactured according to current Good Manufacturing Practice (cGMP), is safe, suitable, and GRAS based on scientific procedures. A summary of the basis of the Expert Panel's conclusion is presented below.

CHEMISTRY AND MANUFACTURING

The subject of this GRAS evaluation is high-purity rebaudioside M (≥85% rebaudioside M), which is a high purified product comprised of ≥85% rebaudioside M and ≥95% total steviol glycosides, consistent with the purity criteria for steviol glycosides as established by JECFA (JECFA, 2017a,b). The remaining 5% of high-purity rebaudioside M (≥85% rebaudioside M) may include additional steviol glycosides. Due to the common steviol backbone, all steviol glycosides share a common metabolic fate in which they are hydrolyzed to steviol in the lower gastrointestinal tract, which is then absorbed into the body, conjugated with glucuronic acid, and eliminated through the urine in humans.

High-purity rebaudioside M (≥85% rebaudioside M) is manufactured using raw materials, processing aids, and equipment that are food-grade ingredients[1], permitted by U.S. regulation, have GRAS status, or have been self-affirmed as safe for use in food for their respective uses. The high-purity rebaudioside M (≥85% rebaudioside M) product is manufactured by an enzymatic bioconversion process using enzymes (2 glucosyltransferases and sucrose synthase) derived from genetically modified *E. coli* strains derived from *E. coli* K-12. High-purity rebaudioside M (≥85% rebaudioside M) is manufactured in a multi-stage process; in the first stage, the starting material, a steviol glycoside mixture containing ≥95% total steviol glycosides is produced in accordance with the methodology outlined in the Chemical and Technical Assessment (CTA) for steviol glycosides as published by the Food and Agriculture Organization of the United Nations (FAO)/JECFA (FAO, 2016). The manufacturing process for the starting material is also described in GRAS Notice (GRN) 275 in which the U.S. FDA had "no questions" regarding its GRAS status (U.S. FDA, 2009). In the second stage, the production strains carrying the expression vector for each corresponding enzyme (glucosyltransferase or sucrose synthase) undergo a fermentation step and downstream processing to generate the enzymes required for the enzymatic bioconversion reaction. In the final stage, the steviol glycosides are mixed with the glucosyltransferases and sucrose synthase enzymes generated from the fermentation step to initiate the enzymatic bioconversion reaction, in which the steviol glycosides are converted to rebaudioside M. The resulting steviol glycoside mixture is purified in a series of filtration and washing steps to yield a final product containing ≥85% rebaudioside M and ≥95% total steviol glycosides.

The Expert Panel reviewed information pertaining to the construction of the enzyme production strains used to generate the glucosyltransferases and sucrose synthase enzymes required for the enzymatic bioconversion reaction. The synthetic genes encoding for each of the 3 enzymes were based on the respective native sequences and are not associated with any known allergens or toxins. The production strains are derived from *E. coli* K-12, which is a non-pathogenic and non-toxigenic organism that has been classified as a Risk Group 1 organism according to the National Institute of Health (NIH) (NIH, 2016).

Appropriate food-grade product specifications have been established for high-purity rebaudioside M (≥85% rebaudioside M) based on the steviol glycosides specification established by JECFA (JECFA, 2017a,b). The steviol glycoside content of high-purity rebaudioside M (≥85% rebaudioside M) is measured using the high-performance liquid chromatography (HPLC) method described in the JECFA specification monograph for steviol glycosides from *Stevia rebaudiana* Bertoni (JECFA, 2017a,b). Review by the Expert Panel of the analytical data for 3 non-consecutive lots of high-purity rebaudioside M (≥85% rebaudioside M) confirms that the final product is produced in compliance with the established product specifications. In addition, residual protein in the final product was confirmed to be below the detection level in 3 non-consecutive lots of high-purity rebaudioside M (≥85% rebaudioside M) *via* the bicinchoninic acid (BCA) assay, and the

[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).

absence of residues of commonly used pesticides in the final product was confirmed in 1 lot of the starting material (>95% total steviol glycosides).

JECFA has concluded that steviol glycosides are thermally and hydrolytically stable for use in foods and acidic beverages under normal processing and storage conditions (JECFA, 2007). To confirm that these conclusions can be extended to high-purity rebaudioside M (≥85% rebaudioside M), Tate & Lyle conducted a storage stability test for up to 36 months on a previous product, TASTEVA® Stevia Sweetener, which is comprised of ≥75% rebaudioside A and stevioside and ≥95% total steviol glycosides, and an accelerated stability study on high-purity rebaudioside M (≥85% rebaudioside M) for up to 6 months. The results of the storage stability study on TASTEVA® Stevia Sweetener are consistent with JECFA's conclusions regarding individual steviol glycoside stability, and therefore can be extended to support the storage stability of high-purity rebaudioside M (≥85% rebaudioside M). The accelerated stability study on high-purity rebaudioside M (≥85% rebaudioside M) is currently on-going; however, the 1-month results indicate that the total steviol glycoside and rebaudioside M content is stable when kept in commercial packaging at 50°C.

INTENDED FOOD USES AND ESTIMATED INTAKE

High-purity rebaudioside M (≥85% rebaudioside M) is intended for use as a general purpose sweetener that will be added to various food and beverage products that are consistent with the current uses of other high-intensity sweeteners on the U.S. market. The estimated intakes of high-purity rebaudioside M (≥85% rebaudioside M) were calculated using a post-market surveillance approach as described by Renwick (2008). The estimated intakes were calculated by adjusting the post-market surveillance data for other high-intensity sweeteners using the sweetness intensity of high-purity rebaudioside M (≥85% rebaudioside M) relative to sucrose (*i.e.,* approximately 208). The results are shown in Table 1. For non-diabetic adults, average and high-end intakes of high-purity rebaudioside M (≥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 (≥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 (≥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 (≥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. Recently, JECFA re-assessed the dietary exposure to steviol glycosides using different intake models, including the approach described by Renwick (2008), and noted that the replacement estimates were highly conservative and that actual exposures to steviol glycosides, expressed as steviol equivalents, would range from 0.4 to 7.2 mg/kg body weight/day (FAO, 2016). JECFA made note that this method overestimates dietary exposure (FAO, 2016).

Table 1 Estimated Consumption High-Purity Rebaudioside M (≥85% Rebaudioside M) Produced by Enzymatic Conversion of Steviol Glycosides 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 for:			
			High-Purity Rebaudioside M (≥85% Rebaudioside M)[a] (mg/kg bw/day)		High-Purity Rebaudioside M (≥85% Rebaudioside M)[b] as steviol equivalents (mg/kg bw/day)	
	Average Consumer	High Consumer	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 rebaudioside M (1,291.3 g/mol) [steviol conversion factor of 0.25].

INFORMATION TO ESTABLISH SAFETY

The Expert Panel reviewed the available data supporting the safety of individual steviol glycosides to evaluate the safety of high-purity rebaudioside M (≥85% rebaudioside M). The available data included a discussion on the metabolic fate of steviol glycosides, a summary of the extensive conclusions on the safety of steviol glycosides by global scientific and regulatory authorities/bodies, including the U.S. FDA, and other data that was deemed pivotal in determining the safety of high-purity rebaudioside M (≥85% rebaudioside M), such as information regarding the safety of the production strains used to produce the glucosyltransferases and sucrose synthase enzymes.

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 1 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 rate 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). Thus, due to the shared metabolic fate of steviol glycosides, the safety database that has been established for individual steviol glycosides (*e.g.,* stevioside, rebaudioside A, rebaudioside D), can be extrapolated to support the safety of purified steviol glycosides in general, regardless of the steviol

glycoside distribution of the preparation, including high-purity rebaudioside M (≥85% rebaudioside M) produced by enzymatic conversion of steviol glycosides.

The safety of steviol glycosides have been extensively reviewed by various scientific and regulatory authorities/bodies, such as JECFA, U.S. FDA, Food Standards Australia New Zealand (FSANZ), the European Commission's Scientific Committee on Food (SCF), the European Food Safety Authority (EFSA), and Health Canada (SCF, 1985, 1999; FSANZ, 2008; EFSA, 2010, 2015; Health Canada, 2012b; JECFA, 2006, 2017a,b). Based on safety data and information that were generally available in the published scientific literature, these scientific bodies and regulatory agencies have unanimously concluded that steviol glycosides is of no safety concern and have established an ADI of 0 to 4 mg/kg body weight, expressed as steviol equivalents, based on the results of a 2-year study in rats in which no carcinogenicity or adverse effects on any study parameter were observed (Toyoda *et al.,* 1997). The no-observed-adverse-effect level (NOAEL) of 970 mg/kg body weight/day, equivalent to 383 mg/kg body weight/day as steviol, determined from the results of the study by Toyoda *et al.* (1997) became the basis for the established ADI following application of a safety factor of 100 (JECFA, 2009; FSANZ, 2008; EFSA, 2010; Health Canada, 2012b). Subsequent to these safety evaluations, the 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, 2017b; Health Canada, 2017; JECFA, 2017a,b).

The U.S. FDA has reviewed the safety of over 50 different steviol glycoside preparations and have consistently raised no objections regarding the GRAS status of steviol glycosides for use as general purpose sweeteners in food and beverage products. Of note, the U.S. FDA did not raise any objections regarding GRN 667, which describes the GRAS status of rebaudioside M produced by an enzymatic bioconversion process for use as a general purpose sweetener in foods (U.S. FDA, 2016). The rebaudioside M described in GRN 667 is similar to Tate & Lyle's high-purity rebaudioside M (≥85% rebaudioside M) produced by enzymatic conversion of steviol glycosides in that the food ingredient is produced from enzymatic conversion of stevia leaf extract using UDP-glucosyltransferase and sucrose synthase enzymes derived from microorganisms that have been genetically modified to produce these enzymes.

A comprehensive search of the scientific literature was conducted through March 2018 to capture publications relevant to the safety of steviol glycosides that became available following the U.S. FDA review of GRN 733[2]. The search identified studies investigating the genotoxicity, antidiabetic, and immune effects of steviol glycosides. Upon review, the Expert Panel noted that the results of these studies do not contradict the safety conclusions on steviol glycosides as established by a number of scientific and regulatory authorities/bodies (*e.g.,* JECFA, FSANZ, U.S. FDA, EFSA, and Health Canada), and therefore provide further support for the safety of steviol glycosides.

The parental strain, *E. coli* K-12, from which the production strains are derived, has an extensive history of use as a laboratory organism and is considered one of the most extensively characterized microorganisms (Bachmann, 1972; Jensen, 1993). *E. coli* K-12 has a long history of safe use in the food and pharmaceutical industries. For example, a genetically modified strain of *E. coli* K-12 producing chymosin was affirmed as GRAS by the U.S. FDA in 1990 (Flamm, 1991; Olempska-Beer *et al.*, 2006) and 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). Further, *E. coli* K-12 and its derivatives are unable to colonize the mammalian gastrointestinal tract, and do not produce toxins and are

[2] At the time of the Expert Panel's evaluation, GRN 733 was the most recent GRAS notice reviewed by the U.S. FDA to receive a "no questions" letter.

unable to persist in the soil and water (Bogosian *et al*., 1996; U.S. EPA, 1997). 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 Expert Panel reviewed the potential allergenicity and pathogenicity of the enzymes using an *in silico* approach. The allergenicity potential of the enzymes was investigated using the approach described in the FAO/World Health Organization (WHO) protocol for allergenicity assessment (FAO/WHO, 2001) and WHO/FAO (2009) using the AllergenOnline Database Version 18 (FARRP, 2018). The database contains a comprehensive list of putative allergenic proteins developed *via* a peer-reviewed process for the purpose of evaluating food safety. No structural similarity greater than 35% to known allergen sequences was identified, indicating the enzymes used in the enzymatic bioconversion process would not be associated with an allergenic response. The potential pathogenicity of the enzymes was investigated in a search with prediction software MP3 tools and a Support Vector Machine (SVM), Hidden Markov Model (HMM), or integrated SVM-HMM approach to predict pathogenic proteins in both genomic and metagenomic datasets. All enzymes were predicted to be non-pathogenic using each SVM, HMM, or integrated SVM-HMM approach. The safety of the glucosyltransferases and sucrose synthase enzymes was evaluated using the Pariza and Johnson (2001) decision tree for evaluating the safety of microbially-derived food enzymes. The enzymes were "accepted" on the basis that the final high-purity rebaudioside M (≥85% rebaudioside M) produced by enzymatic conversion of steviol glycosides product meets the established JECFA specifications, and that the enzymes are absent in the final product. The Expert Panel noted that the manufacturing process includes a heat treatment step in which the enzymes are denatured, and subsequent filtration steps to remove the enzymes to produce a high-purity final product. The Expert Panel reviewed analytical data demonstrating residual protein that could potentially carry over from the enzymatic bioconversion step was not detected and concluded that the potential allergenicity of the enzymes should not be a health concern.

The scientific evidence reviewed by the Expert Panel demonstrates that under the conditions of intended use, high-purity rebaudioside M (≥85% rebaudioside M) would not produce any adverse health effects.

CONCLUSION

We, the members of the Expert Panel, have, independently and collectively, critically evaluated the data and information summarized above and conclude that high-purity rebaudioside M (≥85% rebaudioside M), meeting appropriate food-grade specifications and produced in accordance with current Good Manufacturing Practice (cGMP), is safe for use as a general purpose sweetener in foods and beverages.

We further unanimously conclude that the proposed use of Tate & Lyle's high-purity rebaudioside M (≥85% rebaudioside M) meeting appropriate food-grade specifications, as presented in the supporting dossier and produced consistent with cGMP is Generally Recognized as Safe (GRAS) under its intended conditions of use as a general purpose sweetener in food and beverages based on scientific procedures.

It is our opinion that other qualified experts would concur with these conclusions.

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