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Application for the Approval of Rebaudioside M from *Saccharomyces cerevisiae* expressing Steviol Glycoside Biosynthesis Pathway Genes

Executive Summary

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EXECUTIVE SUMMARY

Amyris Inc. is submitting this application to Food Standards Australia New Zealand (FSANZ) to request amendment to the Australia New Zealand Food Standards Code (the Code) to permit the use of steviol glycosides rebaudioside M (Reb-M), produced by fermentation from *Saccharomyces cerevisiae* (*S. cerevisiae*) that expresses steviol glycoside biosynthesis pathway genes, as a general-purpose sweetening agent. Amyris' steviol glycoside product is composed of no less than 95% Reb-M with other steviol glycosides being present at trace amounts. The Code currently permits steviol glycosides to be used as intense sweeteners in a variety of foods (Standard 1.3.1 – Food Additives and Schedule 15 – Substances that may be used as Food Additives).

Up until recently, only the leaf of *Stevia rebaudiana* (*S. rebaudiana*) was permitted to be a source of steviol glycosides. However, Schedule 3 – Identity and Purity of the Code was recently amended to include a specification for permitted steviol glycosides to be produced from fermentation of a *S. cerevisiae* strain (CD15407) that expresses steviol glycoside biosynthesis genes (S3—39). Amyris' steviol glycoside product meets all but one aspect of the S3—39 specification; the specific production strain of *S. cerevisiae* is not the same as that listed in the Code and is therefore not permitted.

Reb-M is associated with an improved sensory profile when compared to major leaf-derived steviol glycosides, such as rebaudioside A and stevioside. Recently developed manufacturing methods, including fermentation, are much more efficient in producing steviol glycosides, such as Reb-M that are present in only small quantities in the traditional leaf extractions of *S. rebaudiana*. Amyris' Reb-M is produced via fermentation of sugar by a strain of *S. cerevisiae* that has been modified to contain genes from the pathway in the *S. rebaudiana* plant that produces steviol glycosides. *S. cerevisiae* has a very long history of safe use in foods

The safety of steviol glycosides has been subject to extensive assessment by international and national agencies, such as FSANZ, the FAO/WHO Joint Expert Committee on Food Additives (JECFA), the European Food Safety Authority and Health Canada. The United States Food and Drug Administration (FDA) has responded with no questions to Amyris' generally recognised as safe (GRAS) notification for its Reb-M produced from *S. cerevisiae*. The FDA has similarly responded with no questions in relation to numerous other steviol glycoside GRAS notifications (for various types and production methods of steviol glycosides).

FSANZ has previously concluded that steviol glycosides, including Reb-M, are safe for human consumption when added to foods at the permitted use levels listed in Schedule 15 of the Code. JECFA, at its 87th meeting (2019) agreed that the acceptable daily intake (ADI) for leaf-derived steviol glycosides established by JECFA at the 69th meeting (2008) also apply to steviol glycosides produced by four other methods (including fermentation). This is the ADI that FSANZ has used in its previous safety assessments of steviol glycosides. Amyris is not requesting any change to the current permissions in Schedule 15. In addition, Amyris' Reb-M will be used as a replacement for other steviol glycosides, so expected dietary intake of Reb-M is expected to be similar to the dietary intake of other steviol glycosides already used in foods in Australia and New Zealand. Therefore, previous safety assessments, including estimated dietary intakes, remain relevant to the intended use of Amyris' Reb-M product in foods.

Amyris' Reb-M production organism is a strain of the yeast *S. cerevisiae*. *S. cerevisiae*, commonly known as brewer's yeast and baker's yeast, is well characterised and has an extensive history of safe use in the food industry around the world, including in Australia and New Zealand. The production strain of *S. cerevisiae* used by Amyris has been genetically modified by adding genes necessary to produce steviol glycosides, predominately Reb-M. The Amyris production strain contains no known pathogenicity-related proteins, toxins, allergens or pyrogens. In addition, all the heterologous genes introduced into the production strain are derived synthetically from biosafety level 1 organisms and are codon optimized for expression in *S. cerevisiae*. The heterologous genes are not associated with any known allergens or toxins.

Following fermentation and the production of Reb-M, the fermentation broth is subject to heat treatment to kill the yeast cells prior to purification/concentration steps, which removes the production organism from the Reb-M product. Therefore, the final Reb-M product is a highly purified product that contains no residual source organism or introduced DNA. The absence of protein and residual DNA in the final Reb-M product is confirmed by analytical testing.

Amyris' Reb-M is a highly purified product produced via fermentation by a genetically modified strain of *S. cerevisiae* that expresses steviol glycoside biosynthesis pathway genes. FSANZ's recent amendment of the Code to permit steviol glycosides produced by fermentation by genetically modified *S. cerevisiae* and JECFA's recent consideration of alternative production methods of steviol glycosides (JECFA87) acknowledge that fermentation by genetically modified organisms produces steviol glycosides that are equivalent to traditionally produced leaf-extracted steviol glycosides. The information provided to support this application, coupled with the extensive assessment of the safety of steviol glycosides, including Reb-M, by FSANZ and other agencies internationally demonstrates that Reb-M produced via fermentation by a genetically modified strain of *S. cerevisiae* expressing steviol glycoside biosynthesis pathway genes is safe for consumption when used in accordance with the permission for steviol glycosides in Schedule 15 of the Code.