# Application for the Approval of Rebaudioside M under Australia and New Zealand Food Standard Code Standard 1.3.1– Food Additives and Standard 1.3.4 – Identity and Purity

# EXECUTIVE SUMMARY

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Steviol glycosides are currently approved for use as a food additive by the Food Standards Australia New Zealand (FSANZ) under section 1.3 – Substances Added to Food of the Australia New Zealand Food Standards Code (*The Code*). Under this regulation, steviol glycosides are permitted for use as intense sweeteners and are considered safe for inclusion in food provided they are used at levels at or below that outlined in the regulation (FSANZ, 2014a,b,c).

Rebaudioside M, also known as rebaudioside X, has been identified as a minor steviol glycoside within the *Stevia rebaudiana* Bertoni (*S. rebaudiana*) plant. Currently available commercial preparations of stevia extracts have been shown to contain measurable levels of rebaudioside M at levels of up to 0.26% (Prakash *et al*., 2013). Commercially available steviol glycoside products presently permitted for use in food in Australia and New Zealand must comply with the existing steviol glycoside specifications established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (JECFA, 2010), which stipulate not less than 95% stevioside, rebaudioside A, B, C, D, and F, steviolbioside, rubusoside, and dulcoside on a dried basis. Notably, rebaudioside M is presently not listed as one of the steviol glycosides that may be included with other steviol glycosides to make up the set assay limit of at least 95% total steviol glycosides.

Approval for the use of rebaudioside M for use as an intense sweetener in food and beverage applications in Australia and New Zealand is sought by PureCircle Limited (hereafter PureCircle). Rebaudioside M is one of several naturally occurring glycosylated derivates of steviol. In comparison to rebaudioside A, it contains 2 additional β-D-glucosyl moieties at the C-2' and C-3' positions. Consistent with the use of already permitted steviol glycoside preparations, preparations containing rebaudioside M are intended for use as natural, low-calorie, high-intensity sweeteners that offer numerous technological advantages and benefits to consumers and are suitable for use by individuals with diabetes, as well as others who follow a low-glycaemic diet. However, in comparison to existing steviol glycosides, rebaudioside M-containing preparation provide greater sweetness potency and better taste properties. Steviol glycoside preparations rich in rebaudioside M are produced in accordance with current Good Manufacturing Practices (cGMP) and meet appropriate food-grade specifications. Like the production process described for steviol glycoside preparations already available for sale in Australia and New Zealand, the production process for PureCircle's rebaudioside M-containing preparation consists of hot-water extraction of *S. rebaudiana* leaves, followed by extensive step-wise purification and repeated crystallisation of the primary steviol glycoside extract to obtain a preparation that contains at least 50% rebaudioside M, up to ≥95%. Rebaudioside M-containing preparations are intended for use as a sweetener in the same food categories and at the same use-levels already permitted for other steviol glycoside products [as outlined in *The Code* under Section 1.3 (FSANZ, 2014a,b,c)].

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Analysis of rebaudioside M-rich preparations confirms that with the exception of the assay value, which presently cannot be met given the absence of rebaudioside M from the list of steviol glycosides that may contribute to the assay value; all other parameters of the current specifications for steviol glycosides are met. Furthermore, both the high rebaudioside M content preparation (≥95% rebaudioside M), as well as products with a lower rebaudioside M content (of at least ≥50%) consistently met the requirement of not less than 95% total steviol glycosides. Considering that rebaudioside M shares a common chemical structure and that as all other already recognised steviol glycosides has been confirmed to be subject to similar metabolic processes (*i.e.*, hydrolysis to steviol and component sugar moieties), it is reasonable to conclude that the existing acceptable daily intake (ADI) for steviol glycosides which is expressed on the basis of the common metabolite steviol is equally applicable to rebaudioside M. As such, there does not appear to be any reasonable justification for why rebaudioside M should not be permitted for use as an intense sweetener in food and beverage applications, particularly since steviol glycoside preparations rich in rebaudioside M have been shown to possess taste qualities that are superior to existing steviol glycoside preparations. Therefore, the use of rebaudioside M will in fact be of benefit to consumers of steviol glycoside preparations in Australia and New Zealand. Furthermore, the established specifications for steviol glycosides were developed to cover a range of preparations, and while up until now rebaudioside A and stevioside have been recognised as the glycosides of primary interest with respect to sweetening properties of steviol glycoside preparations, rebaudioside M appears to provide greater sweetness intensity than either rebaudioside A or stevioside and a superior taste profile.

As already indicated, given the structural similarities and metabolic fate of rebaudioside M and other common steviol glycosides, data previously considered in support of the safety of steviol glycosides, can also be relied upon to support the safety of rebaudioside M. Safety data that were already reviewed by a number of regulatory agencies in relation to their evaluation of steviol glycoside safety was not re-evaluated as part of this food additive application. However, a search of the scientific literature was conducted to determine whether any new data related to the safety of steviol glycosides since 2010 (year of the most recent steviol glycoside assessment by FSANZ). The literature search did not reveal any additional information that would in any way raise questions regarding the established safety conclusions related to steviol glycosides in general, and hence rebaudioside M. In fact, in addition to the study which confirmed the hydrolysis of rebaudioside M to steviol, a few other *in vitro* and *in vivo* studies were identified with related steviol glycosides (rebaudioside D, B, and E), which also showed that as expected these glycosides are primarily hydrolysed to steviol in the colon prior to absorption.

With respect to studies addressing safety, one sub-chronic toxicity study was identified through the literature search, in which some questionable liver effects were noted in animals administered an aqueous extract of the *S. rebaudiana* leaf. Considering however that the purity of the extract was not reported, it is not possible to ascertain that the test article provided met

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the specifications for high-purity steviol glycosides (≥95% purity), and therefore the study is considered to be of limited value in the assessment of the safety of highly purified steviol glycoside preparations. Additionally, this study was of poor quality with regard to testing protocol, reporting details, and evaluation of the results by the investigators.

A second, non-Good Laboratory Practice-compliant sub-chronic toxicity study involving immature rats was conducted with a stevioside product that was reported to meet current JECFA specifications for steviol glycosides (*i.e.*, ≥95% purity). The investigators reported that no adverse effects were observed in animals fed the low dose of 15 mg stevioside/kg body weight/day; however, various haematological parameters [increased lipids, reduced alkaline phosphatase (ALP)/ tartrate-resistant acid phosphatase (TRAP)], body weights, and relative weights of kidneys, brain, and tests/epididymis were significantly affected by the administration of stevioside at the higher dose level (1,500 mg/kg body weight/day). Reduced body weights in animals fed high-intensity sweeteners is a well-known side-effect of these diets and is likely related to palatability and reduced nutritional quality of the diet and as such is not considered to be an adverse effect. Furthermore, the reduced body weights in the high-dose group may account for the increases observed in relative organ weights, particularly since no significant increases in the absolute weights of the affected organs were reported. With respect to the variability observed in certain haematological parameters in this study, notable peculiarities about the values have raised questions about the analytical veracity of the reported results. Specifically, comparison of the values obtained in control animals to available reference values for some of the affected parameters such as haemoglobin and mean corpuscular volume, suggests that the animals used in this study were anaemic at testing outset; however, no clinical signs indicative of anaemia were reported in the study. Considering that the haematological variability which would indicate a state of anaemia was unparalleled by corresponding clinical observations, the accuracy of the results is questionable. Furthermore, the conclusion derived by the study authors that related the observed reductions in ALP and TRAP value to adverse effects on bone mineralisation was poorly substantiated considering that the techniques for measuring TRAP were out-dated and not specific to bone. Finally, it should be noted that even if the results of this study were to be interpreted as being related to the administration of stevioside, the dose level of 1,500 mg/kg body weight/day which would be associated with these effects, is greater than the no-observed-adverse-effect level of 970 mg/kg body weight/day previously determined for stevioside and which formed the basis for the currently set ADI of up to 4 mg/kg body weight/day (expressed as steviol equivalents) for steviol glycosides.

Furthermore, in a GLP-, OECD-, and FDA Redbook-compliant 28-day study identified through the literature search, no adverse effects were observed in rats following the administration of rebaudioside D in the diet at concentrations providing dose levels of up to approximately 2,000 mg/kg body weight/day. This study also additionally incorporated a group of rats which was provided rebaudioside A at a concentration providing a similar dose level. The absence of any adverse effects following parallel administration of either steviol glycoside, combined with

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the toxicokinetic results obtained in this study, further support that safety data obtained for one steviol glycoside may on the basis of similar metabolic pathways be applied in a safety evaluation of a related steviol glycoside.

All other information identified in the literature search provided further reassurance of the safety of steviol glycosides for human consumption.

Overall, the data provided supports the conclusion that use of steviol glycoside preparations containing rebaudioside M in foods and beverages for human consumption at the use-levels presently permitted in Australia and New Zealand for steviol glycosides does not present a significant risk to human health and is safe. In fact, use of rebaudioside M-containing preparations provides technological benefits that may not be presently achieved with the use of currently available steviol glycoside preparations. Considering that rebaudioside M is related to the 9 other already recognised steviol glycosides, occurs naturally in the *S. rebaudiana* Bertoni plant, and can be extracted from the plant resulting in steviol glycoside preparations that comprise ≥50% rebaudioside M, the use of rebaudioside M as a high-intensity sweetener in food and beverage applications does not present a safety concern and is justified.

## REFERENCES


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