

**Application for the Approval of Those Steviol Glycosides
Present in *Stevia rebaudiana* under Australia and New
Zealand Food Standard Code Standard 1.3.1– Food
Additives**

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 Part 1.3 – Substances Added to or Present in Food of the Australia New Zealand Food Standards Code (*The Code*). Under *The Code*, 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 Schedule 15 (FSANZ, 2016). Currently as listed in Schedule 3 – Purity and Identity of *The Code*, steviol glycosides are a mixture comprising not less than 95% of the 10 named steviol glycosides, which include rebaudioside A, rebaudioside B, rebaudioside C, rebaudioside D, rebaudioside F, rebaudioside M, stevioside, dulcoside A, rubusoside, and steviolbioside. All other parameters relating to the identity and purity of steviol glycosides are consistent with those standards established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), the European Commission, and the Food Chemical Codex (FCC).

The number of steviol glycosides that have been identified in the *Stevia rebaudiana* plant has now increased to approximately 40, including the 10 above named steviol glycosides (major steviol glycosides) that currently are permitted for use in steviol glycoside preparations in Australia and New Zealand. Presently, PureCircle Limited (hereinafter “PureCircle”) is seeking to expand the definition of steviol glycosides to include all steviol glycosides present in *Stevia rebaudiana* (*S. rebaudiana*) (*i.e.*, steviol conjugated with glucose, rhamnose, xylose, fructose, deoxyglucose and/or other sugar moieties in any orientation, quantity, or combination) and gain approval for the use of SG mixtures for use as an intense sweetener in food and beverage applications in Australia and New Zealand. Consistent with the use of already permitted steviol glycoside preparations, SG mixtures 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 glycoside preparations containing only major steviol glycosides, SG mixtures provide improved flavour and taste characteristics in various foods.

SG mixtures 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 SG mixtures 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 95% steviol glycosides. Because different sections of the column system used during the manufacture of SG mixtures adsorb different proportions of steviol glycosides and these different sections are desorbed separately, SG mixtures with different ratios of steviol glycosides can be produced simultaneously. Therefore, the steviol glycoside distribution is specific to each individual SG mixture and could contain any steviol glycoside identified in the *S. rebaudiana* plant in any proportion. Analyses of SG mixtures confirm that with the exception of the assay value, which presently cannot be met given the absence of the minor

steviol glycosides from the list of steviol glycosides that may contribute to the assay value, all other parameters of the current specification requirements for steviol glycosides are met. Furthermore, example commercial preparations of SG mixtures (*i.e.*, RA50 and A95) consistently met the requirement of not less than 95% total steviol glycosides, with approximately 5% of the assay value being represented by the additional minor steviol glycosides.

Considering these minor steviol glycosides share a common chemical structure and that steviol glycosides have 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 expressed on the basis of the common metabolite steviol, is equally applicable to all steviol glycosides. In order to corroborate previous conclusions regarding the shared metabolic pathway for steviol glycosides, studies comparing the microbial metabolism and hydrolysis rates of various steviol glycosides (rebaudioside A, B, C, D, E, F, M, steviolbioside, dulcoside A, and fructosylated rebaudioside A) *in vitro* were conducted. Based on the findings of these studies, it was deemed appropriate to extend the safety conclusions for steviol glycosides to all glycosylated derivatives of the aglycone steviol (purified).

In vitro and *ex vivo* studies have demonstrated that steviol glycosides are not hydrolysed by digestive enzymes of the upper gastrointestinal tract and are not absorbed through the upper portion of the gastrointestinal tract (Hutapea *et al.*, 1997; Koyama *et al.*, 2003a; Geuns *et al.*, 2003, 2007). Steviol glycosides enter the colon intact, where they are subject to microbial degradation by members of the *Bacteroidaceae* family, resulting in the release of the aglycone steviol (Gardana *et al.*, 2003; Renwick and Tarka, 2008). Several *in vitro* studies mimicking the anaerobic conditions of the colon, reviewed extensively by Renwick and Tarka (2008), have confirmed the ability of gut microflora from mice, rats, hamsters, and humans to hydrolyse steviol glycosides completely to steviol (Wingard *et al.*, 1980; Hutapea *et al.*, 1997; Gardana *et al.*, 2003; Koyama *et al.*, 2003b). Specifically, Koyama *et al.* (2003b) investigated the degradation of a stevia mixture containing rebaudioside A, stevioside, rebaudioside C, and dulcoside A (percent composition not reported) in the presence of human faecal homogenates under anaerobic conditions. Similar to studies conducted with single steviol glycosides, the stevia mixture was degraded completely to steviol within 24 hours of incubation (Koyama *et al.*, 2003b). Rebaudioside E incubated *in vitro* with crude pectinase, an enzyme associated with the resident pectinolytic bacteria of the human intestine, was found to be hydrolysed to steviol (Jensen and Canale-Parola, 1985). More recently, rebaudioside D was incubated with rat caecal contents for 90 minutes and hydrolysis to stevioside and steviol was found to be comparable to that of rebaudioside A (Nikiforov *et al.*, 2013).

In order to confirm the similarities in microbial metabolism among steviol glycosides, several *in vitro* studies comparing the degradation of various steviol glycosides to rebaudioside A in the presence of human faecal homogenates were conducted (Purkayastha *et al.*, 2014, 2015, 2016; Kwok, 2015). The results from these experiments demonstrate a remarkable similarity with respect to the rate of hydrolysis of the individual steviol glycosides to steviol,

particularly during the first 24 hours of incubation, and indicate that the number and location of sugar units attached to the steviol backbone does not significantly impact the rate of hydrolysis. Since none of the steviol glycosides tested demonstrated a significantly faster rate of hydrolysis than rebaudioside A, there is no concern that consumption of any of these compounds would increase the circulating plasma levels of the potentially toxic aglycone metabolite (steviol) relative to those achieved by rebaudioside A. While subtle differences in the rate of individual steviol glycoside hydrolysis may exist, it does not appear that these differences would be great enough to significantly impact the absorption rate of steviol (that could potentially alter the level of systemic metabolism and clearance of steviol). The results of these studies corroborate the findings from previously published studies demonstrating that all steviol glycosides sharing the same steviol backbone are degraded by faecal microbes to steviol in the gastrointestinal tract. Therefore, since the major steviol glycosides as well as the many “minor” steviol glycosides recently identified share a common metabolic fate, the safety database established for steviol glycosides can be extended to all glycosylated derivatives of the aglycone steviol (purified), including the proposed SG mixtures.

Studies comparing the metabolic fate of rebaudioside A and stevioside demonstrate that both glycosides have similar pharmacokinetics in the rat; they are both metabolised in the gut to steviol prior to absorption followed by glucuronidation in the liver and excretion in the faeces *via* the bile (Nakayama *et al.*, 1986; Sung, 2002; Koyama *et al.*, 2003a; Roberts and Renwick, 2008). In both rats and humans, steviol was shown to be metabolised to steviol glucuronide following absorption (Nakayama *et al.*, 1986; Koyama *et al.*, 2003a; Geuns and Pietta, 2004 [unpublished]; Simonetti *et al.*, 2004; Geuns *et al.*, 2007; Roberts and Renwick, 2008; Wheeler *et al.*, 2008). However, in humans, elimination of steviol glycosides, primarily as steviol glucuronide with very small amounts of the unchanged glycoside or steviol, occurs *via* the urine (Kraemer and Maurer, 1994; Geuns and Pietta, 2004 [unpublished]; Simonetti *et al.*, 2004; Geuns *et al.*, 2006, 2007; Wheeler *et al.*, 2008). Circulating steviol glycosides have not been detected in the plasma of humans, nor in the majority of animal studies conducted, indicating that the parent compound is not absorbed into the systemic circulation (Geuns *et al.*, 2003, 2007; Roberts and Renwick, 2008; Wheeler *et al.*, 2008). Overall, the data demonstrate that rebaudioside A and stevioside have similar metabolism and pharmacokinetics in the rat and human.

With the exception of having different numbers and types of sugar moieties, all steviol glycosides share the same steviol backbone and are thus expected to follow the same metabolic pathway as demonstrated for rebaudioside A and stevioside. Given the structural similarities and metabolic fate of steviol glycosides, data previously considered in support of the safety of steviol glycosides, can also be relied upon to support the safety of SG mixtures. Specifically, the safety of steviol glycosides has been the subject of numerous reviews over the last couple of decades, and recently multiple jurisdictions including the United States (U.S.), European Union (EU), Australia and New Zealand, and Canada have concluded that preparations containing at least 95% steviol glycosides are safe when used in accordance with cGMP (FSANZ, 2008, 2015; U.S. FDA, 2008a,b, 2009a-d, 2010a-e, 2011a-i, 2012a-e,

2013a-f, 2014a-c, 2015a-c; EFSA, 2010, 2015; Health Canada, 2012a,b, 2016). The safety of steviol glycosides was reviewed by JECFA at 4 separate meetings (51st, 63rd, 68th, and 69th) in 1998, 2004, 2007, 2008. Based on the similar metabolic pathway for all steviol glycosides in rats and humans, as presented above, JECFA established an ADI of 0 to 4 mg/kg body weight, as steviol equivalents, based on a no-observed-adverse-effect level (NOAEL) of 970 mg/kg body weight/day (383 mg/kg body weight/day as steviol) from a 2-year study in rats (Toyoda *et al.*, 1997) and a safety factor of 100, to account for intra- and inter-species differences (JECFA, 2010). The safety of steviol glycosides is based on the general recognition that all steviol glycosides are degraded to the aglycone steviol and that the safety demonstrated for one glycoside is relevant to all glycosides, and therefore, it was determined that the JECFA's ADI for steviol glycosides also would extend to SG mixtures in general.

SG mixtures 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 Schedule 15 (FSANZ, 2016). As the current steviol glycoside concentrations are provided in terms of steviol equivalents, a conversion factor for all steviol glycosides present in *S. rebaudiana* would normally be required to be provided and added to the conversion factor table listed in *Standard 1.3.1 – Food Additives of The Code*. However, given that SG mixtures may contain any steviol glycoside in any proportion, applying multiple conversion factors to a mixture of steviol glycosides are considered impractical. Therefore, PureCircle proposes to utilise a single conversion factor of 0.33, which is representative of typical SG mixtures and of the median conversion factor for all major steviol glycosides present in the final preparations (0.25 to 0.4), with the minor glycosides typically representing much smaller amounts (approximately 5%) of the final preparation containing all potential steviol glycosides.

Overall, the data provided supports the conclusion that use of SG mixtures 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 SG mixtures provides technological benefits that may not be presently achieved with the use of currently available steviol glycoside preparations. Considering that the additional “minor” steviol glycosides are related to the 10 other already recognised steviol glycosides, occur naturally in the *S. rebaudiana* Bertoni plant, and can be extracted from the plant resulting in steviol glycoside preparations that comprise ≥95% steviol glycosides, the use of SG mixtures as a high-intensity sweetener in food and beverage applications does not present a safety concern and is justified.

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