Application for the Approval of Glucosylated Steviol Glycosides under Australia and New Zealand Food Standard Code – Standard 1.3.1– Food Additives

EXECUTIVE SUMMARY

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PureCircle Limited (PureCircle) is seeking amendment to Standard 1.3.1 and related Schedules to include the use of glucosylated steviol glycosides as a high-intensity sweetener for the replacement of sucrose in reduced-calorie or no-sugar-added products. Glucosylated steviol glycoside preparations are comprised of a mixture of glucosylated steviol glycosides and parent steviol glycosides, and are generated through enzymatic addition of glucose moieties to steviol glycoside extracts (≥95%) obtained from the leaves of *S. rebaudiana* Bertoni. Glucosylated steviol glycosides are not 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*). However, steviol glycosides, the parent compound, are currently approved for use as a food additive by the FSANZ under *The Code*, specifically 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, 2017). Consistent with the use of already permitted steviol glycoside preparations, glucosylated steviol glycosides are intended for use as 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 parent steviol glycosides, the addition of glucose units to parent steviol glycosides to generate glucosylated steviol glycosides improves the sensory characteristics of the glycosides by reducing bitterness and astringency, enhancing the fruit flavours of beverages, and in general improving the overall liking by consumers.

Glucosylated steviol glycoside preparations are comprised of a mixture of glucosylated steviol glycosides, containing 1 to 20 additional glucose units bound to the parent steviol glycoside. Based on distribution analyses, the mono-, di-, and tri-glucosylated forms generally predominate, and the glucosylated fraction will make up approximately 80 to 92% of the total steviol glycoside content and the parent glycosides approximately 5 to 15%. PureCircle's glucosylated steviol glycosides contain not less than 95% total steviol glycosides, determined as the sum of the glucosylated steviol glycosides and the parent steviol glycosides. Physical, chemical and microbiological specifications for glucosylated steviol glycosides are proposed based on those most recently established for 'Steviol Glycosides from *Stevia rebaudiana* Bertoni' by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (JECFA, 2016). The current FSANZ specifications for steviol glycosides as well as those established for rebaudioside A and steviol glycosides published in the Food Chemicals Codex (FCC, 2016a,b) were also referenced. Analysis of 5 non-consecutive lots of a representative commercial preparation of glucosylated steviol glycosides demonstrates that the manufacturing process, as described in the next paragraph, produces a consistent product that conforms to the proposed specification parameters.

Glucosylated steviol glycosides are produced in accordance with current Good Manufacturing Practices and meet appropriate food-grade specifications. The production

process is consistent with the methodologies for the manufacture of steviol glycosides as described in the respective Chemical and Technical Assessment published by JECFA (2007). Like the production process described for steviol glycoside preparations already available for sale in Australia and New Zealand, the production process for PureCircle's glucosylated steviol glycosides begins with hot-water extraction of *S. rebaudiana* leaves, followed by a series of purification and concentration steps, and spray drying to yield a high-purity stevia extract powder of not less than 95% total steviol glycoside content. In the next stage of production, the purified steviol glycosides (≥95%) are enzymatically modified such that additional glucose moieties are conjugated to the parent steviol glycoside structure *via* α-(1-4) linkages by the use of a cyclomaltodextrin glucanotransferase. The enzyme treatment generates a mixture of glucosylated steviol glycosides containing from 1 to 20 additional glucose units bound to the parent steviol glycosides. The enzymes are then inactivated and removed, and the reaction mixture undergoes a series of purification and concentration steps. The refined solution is spray dried and sifted to result in the final glucosylated steviol glycoside powder that consists of not less than 95% total steviol glycosides, made up of a mixture of glucosylated steviol glycosides of different molecular weights as well as any unreacted parent steviol glycosides.

Glucosylated steviol glycosides, also termed enzymatically modified stevia, are approved for use as food additives and/or sweeteners in the United States (U.S.), Japan, Korea, and Malaysia (Government of Malaysia, 2014; Japan Food Chemical Research Foundation, 2014; MFDS, 2015). Specifically, glucosylated steviol glycosides have a safe history of use in Japan for over 25 years. In the U.S., a number of enzyme-modified steviol glycosides preparations are Generally Recognised as Safe (GRAS) for use as flavouring agents and/or general purpose sweeteners in foods (GRN 337, 375, 448, 452, 607, 656, 662) (U.S. FDA, 2011a,b, 2013a,b, 2016a,b,c). Of note, GRN No. 662 was submitted by PureCircle to the Food and Drug Administration (FDA) (U.S. FDA, 2016c) [referred to as ‘’Glucosylated steviol glycosides (minimum purity 95%)" by the FDA] which is equivalent to the glucosylated steviol glycosides presented herein. The FDA raised "No Questions" regarding the GRAS status of PureCircle's glucosylated steviol glycosides (≥95%) described in GRN No. 662 for use as general purpose sweeteners in the U.S. Furthermore, a glucosylated steviol glycoside preparation manufactured by PureCircle, similar to the glucosylated steviol glycosides that are the subject of this evaluation, was also concluded to have GRAS status by the FEMA GRAS Expert Panel in 2010 for use as a flavouring agent (FEMA # 4728) (Marnett *et al.*, 2013; Leffingwell and Leffingwell, 2014).

The scientific conclusions regarding the safety of glucosylated steviol glycosides are primarily based on the fact that glucosylated steviol glycosides and parent steviol glycosides undergo a common metabolic pathway following ingestion. Steviol glycosides (parent as well as glucosylated) are hydrolysed in the large intestine to steviol, and steviol is absorbed and conjugated with glucuronic acid to form steviol glucuronide, which is excreted primarily *via* the urine in humans. The metabolism of glucosylated steviol glycosides has been evaluated in a number of *in vitro* studies, and in the presence of human faecal homogenates, glucosylated steviol glycosides were shown to be hydrolysed to steviol within 24 hours,

comparable to parent steviol glycosides (Koyama *et al.,* 2003). More specifically, the metabolic pathway for α-glucosylated steviol glycosides was shown to start with α-deglucosylation to the parent steviol glycoside, followed by hydrolysis to steviol, similar to that established for parent steviol glycosides (Koyama *et al.,* 2003). These data confirm that glucosylated steviol glycosides and the parent steviol glycosides undergo a common metabolic processing pathway following ingestion (*i.e.* hydrolysis in the large intestine to steviol, followed by absorption and glucuronidation), and therefore, the available data on the parent compound can be used to corroborate the safety of glucosylated steviol glycosides.

The safety of steviol glycosides has been reviewed by several scientific and regulatory authorities including the FDA, JECFA, FSANZ, European Commission's Scientific Committee on Food, European Food Safety Authority (EFSA), and Health Canada. JECFA has evaluated steviol glycosides at 5 previous meetings (51st, 63rd, 68th, 69th and 73rd), and established an acceptable daily intake (ADI) of 4 mg/kg body weight for steviol glycosides, expressed as steviol. Consistent with JECFA's scientific review on the safety of steviol glycosides, EFSA, FSANZ, and Health Canada have established an ADI of 4 mg/kg body weight for steviol glycosides, expressed as steviol equivalents (FSANZ, 2008; EFSA, 2010; Health Canada, 2012). Furthermore, EFSA recently concluded that safety studies conducted with rebaudioside A and stevioside (*i.e.,* individual steviol glycosides) can extend to other steviol glycosides due to the shared metabolic fate (EFSA, 2015).

In addition to the large database supporting the safety of parent steviol glycosides, publically available safety data obtained specifically with glucosylated steviol glycosides was also reviewed in the safety evaluation. The genotoxic potential and subchronic toxicity of an α-glucosylated steviol glycoside product was evaluated in support of a GRAS determination made by Toyo Sugar (GRN 375 - U.S. FDA, 2011b). The outcomes of the *in vitro* and *in vivo* genotoxicity tests were reported to indicate a lack of genotoxicity. In the subchronic study, rats consumed a diet containing α-glucosylated steviol glycosides at concentrations up to 5.0%, equivalent to 1,059 or 1,153 mg steviol equivalents/kg body weight/day for males or females, respectively, for 13 weeks. No adverse effects were observed on any study parameter that could be attributed to the test material. Overall, it was concluded that the α-glucosylated steviol glycosides were safe and well tolerated and that the no-observed-adverse-effect level (NOAEL) in rats for 13 weeks was the highest concentration tested (5%). This study was consistent to short-term and subchronic studies conducted with steviol glycosides in which the NOAEL values also were established at the highest doses tested.

Glucosylated steviol glycosides are proposed for use as intense sweeteners as an alternative to existing steviol glycosides and are intended for use in the same food categories and the same use-levels already permitted for steviol glycosides as outlined in *The Code* under Schedule 15 (FSANZ, 2017). Glucosylated steviol glycosides have an improved sweetness profile over parent steviol glycosides but the sweetness intensity is lower in relative terms; however, no changes in the intended food categories or the maximum use-levels are proposed for glucosylated steviol glycosides expressed on a steviol equivalent basis. As such, intakes of glucosylated steviol glycosides (as steviol equivalents) will be the same as for steviol glycosides, which are already available in the Australian/New

Zealand marketplace. Accordingly, a separate intake assessment for glucosylated steviol glycosides specifically was not performed for the purpose of this food additive application. It should be further noted that the use-levels for steviol glycosides are expressed as steviol equivalents and as such are not specified for any one particular steviol glycoside, but rather are based on the total content of the aglycone, steviol, in the final food product resulting from the addition of any steviol glycoside product meeting the appropriate specifications.

Overall, the data provided supports the conclusion that use of glucosylated steviol glycosides 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 glucosylated steviol glycosides provides technological benefits that may not be presently achieved with the use of currently available steviol glycoside preparations. Considering that the glucosylated steviol glycosides are produced in a similar manner as existing steviol glycoside preparations, and result in glucosylated steviol glycoside preparations that comprise ≥95% total steviol glycosides, the use of glucosylated steviol glycosides as a high-intensity sweetener in food and beverage applications does not present a safety concern and is justified.

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