APPLICATION TO AMEND SCHEDULE 15 OF THE AUSTRALIA NEW ZEALAND FOOD STANDARDS CODE TO ALLOW THE ADDITION OF STEVIOL GLYCOSIDES IN FRUIT DRINKS

EXECUTIVE SUMMARY

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Food Standards Australia New Zealand (FSANZ) under Part 1.3 – Substances Added to Food of the Australia New Zealand Food Standards Code (FSC) permits the use of steviol glycosides as a food additive. Schedule 15 of Standard 1.3.1 – Food Additives allows steviol glycosides to be used as an intense sweetener in food provided they are used at levels at or below stated in the Schedule (FSANZ, 2016). Currently, the FSC allows for steviol glycosides in fruit and vegetable juices and fruit and vegetable juice products to a level of 50mg/kg, low joule fruit and vegetable juice products at 125mg/kg and 200mg/kg for water based flavoured drinks and formulated beverages. This application seeks to amend Schedule 15 to allow the addition of steviol glycosides in 14.1.2.2.1 Fruit Drink at a level of 200mg/kg.

The proposed change would allow beverage manufacturers to create more innovative products with reduced sugar and palatability comparable to fruit juice. Several diluted juice brands are typically achieving up to a 50% sugar reduction using steviol glycosides. These are not eligible to be labelled as fruit drinks, as the FSC does not permit the addition of steviol glycosides to fruit drinks. They are currently categorised as "fruit flavoured drinks", whereas fundamentally these are fruit drinks. It would also allow for more accurate naming of reduced sugar beverages that contain fruit.

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) has defined steviol glycosides as a product obtained from the leaves of *Stevia rebaudiana* Bertoni. Steviol glycosides have the food additive number INS 960 (JECFA, 2005). The steviol glycosides use in fruit drinks would be as an intense sweetener, which would enable the reduction of sugar in fruit drinks. Food grade specifications for steviol glycosides finalised by JECFA require not less than 95% of the total preparation to be comprised of ten named steviol glycosides, on a dried weight basis (JECFA, 2010).

The following production process for steviol glycoside preparations includes extraction from the leaves, filtration of matter, concentration, absorption in a resin exchange, ion exchange purification, further filtration and spray drying or crystallisation.

Specification for steviol glycoside mixtures is outlined in Schedule 3 of the FSC. Detection of steviol glycosides uses reverse-phase high-performance liquid chromatography (RP-HPLC) coupled with tandem mass spectrometry (Kubica *et al*., 2015).

The safety of steviol glycosides has been reviewed by FSANZ several times (FSANZ, 2008, 2011, 2015). The previous FSANZ safety evaluations examined toxicology literature which focuses on the potential mutagenicity and genotoxicity of steviol glycosides. Overall, *in vitro* and *in vivo* studies have consistently demonstrated that stevioside and rebaudioside A, are not mutagenic or genotoxic (reviewed by Brusick, 2008, and Urban et al., 2013). The single exception, an *in vivo* study by Nunes *et al.* (2007), had significant methodological limitations that led experts and regulators to conclude that the study is irrelevant to the health and safety of high purity steviol glycosides in animals or humans (Brusick, 2008; JECFA, 2008; EFSA, 2010; Urban *et al.*, 2013).

A study by Williams and Burdock, 2009 reported on a battery of *in vitro* and *in vivo* genotoxicity tests of 95.6% pure rebaudioside A. The *in vitro* assays included the Ames test (OECD #471), the mammalian chromosome aberration test (OECD #473), and the mouse lymphoma test (OECD #476). Rebaudioside A produced no positive mutagenic effects in any of the assays at concentrations as high as 5 mg/plate.

The two *in vivo* assays conducted were the mouse micronucleus test (OECD #474) and the unscheduled DNA synthesis (UDS) test in rats (OECD #486). For the micronucleus test, a single intraperitoneal injection was administered to mice in three dose groups. Animals in the highest dose group (750 mg rebaudioside A/kg bw) exhibited some signs of toxicity, though no cytotoxicity was evident. No statistically significant genotoxic effects were observed in any rebaudioside A dose group relative to vehicle control, while positive control animals (administered cyclophosphamide) demonstrated an increase in the incidence of micronucleated immature erythrocytes. In the UDS test, rats were administered a single oral gavage dose of 2 g/kg bw. Per OECD guidelines, hepatocytes were collected after 2-h and 16-h. No toxicity or genotoxicity was reported for any of the rebaudioside A treated animals, while both positive control animals (administered dimethylnitrosamine and 2-acetylaminofluorene) elicited a significant increase in hepatocyte UDS at their respective sampling times.

No additional studies on steviol glycoside acute toxicity in animals have been published since the previous FSANZ safety evaluations. However, four unpublished acute toxicology studies on rebaudioside A (98% purity) conducted by Eurofins/Product Safety Laboratories were included in a 2012 FDA GRAS notification (U.S. FDA, 2012). Oral (0.233 – 5 g/kg) and dermal (2 g/kg) rebaudioside A exposures in rats produced no acute toxicity effects. In addition, rebaudioside A did not elicit primary skin irritation (0.5 g) or primary eye irritation (0.04 g) in rabbits upon dermal or ocular exposures. These results support the prior findings in the published literature that steviol glycosides are not acutely toxic in laboratory animals.

A single subacute animal assay was conducted as a bridging toxicity study to investigate whether previous toxicity studies on rebaudioside A would be appropriate to support the safety evaluation of rebaudioside D. The study by Nikiforov *et al.* (2013) included the oral administration of 0, 500, 1000, or 2000 mg/kg/day rebaudioside D, or 2000 mg/kg/day rebaudioside A, to five groups of 20 Crl:CD(SD) rats respectively, for 28 consecutive days. There were no adverse changes observed in clinical observations, terminal body weights, organ weights, or food consumption, or any remarkable differences in hematological, serum chemistry or urinalysis endpoints between control animals and those administered either rebaudiosides A or D. With one exception, functional observational battery and motor activity endpoints were not impacted by either steviol glycoside at tested doses relative to control animals. The females in all rebaudioside D dose groups had significantly lower ambulatory activity relative to control, though the authors hypothesised that this was the result of quicker habituation of these animals, and not related to treatment. In fact, no differences in ambulation were observed in the highest dose Reb D and Reb A treatment group females, and no differences were reported in any of the treatment group males relative to control group males. Nikiforov *et al.* (2013) concluded that the study was appropriate as a bridging study for rebaudioside D, and that it verified the safety of rebaudioside D for human consumption.

Clinical studies have demonstrated that steviol glycosides are well tolerated in humans and are not associated with adverse effects in healthy humans as well as individuals with type-2 diabetes or who are hypotensive. In addition to the clinical research summarised in the previous safety evaluations, it should be noted that a recent study was published that compared the impact of pre-meal ingestion of stevia with that of other sweeteners on food intake, satiety, and postprandial glucose and insulin levels in healthy lean and obese individuals between the ages of 18-50 (Anton *et al*., 2010). The study reported that stevia significantly lowered post-meal glucose levels relative to sucrose preloads, and significantly lowered post-meal insulin levels compared to both aspartame and sucrose preloads. However, critical

study limitations suggest that the results of this study are not likely to influence the current regulatory position regarding the safety of steviol glycoside in humans.

Based on the general nature of cordial and fruit drinks, a beverage based on water, fruit and sugar. It could reasonably be assumed that the pattern of consumption of these beverage types would be similar, whereby an individual would select one or the other, not both at the same time. In an equivalent manner, 'cordials' are beverages typically based on concentrated fruit juices, water and sugar; the same principle is likely to apply to consumption patterns. In addition, in several of tables within the FSANZ Consumption of Intense Sweeteners in Australia and New Zealand: Benchmark Survey 2003, intakes of 'cordials' and 'fruit drinks' were presented together, likely due to the similar pattern of consumption (FSANZ, 2004). There was no definition provided in the report for this food category, it is noted that the questionnaire provided to participants included examples such as 'Ocean Spray Litestyle', 'Cranberry Classic' and 'Sunraysia Diet Lemon Squash' to represent 'fruit drinks'.

The dietary exposure estimates are sufficiently conservative to support the proposed extension of use of steviol glycosides to fruit drinks at 200 mg/kg without resulting in an expected change in the pattern of consumption. As opposed to an increase in the total estimated dietary exposure levels by the total Australian and New Zealand populations.

The review of most recent additions to literature along with the large previously reviewed database on steviol glycosides supports the use of steviol glycosides in fruit drinks at 200mg/kg as safe for human consumption. This would allow greater innovation for industry and provide lower sugar alternatives to consumers.

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