FOOD STANDARDS
Australia New Zealand
Te Mana Kounga Kai – Ahitereiria me Aotearoa

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[54-18]

Supporting document 1

Risk and technical assessment report – Application A1129

Monk fruit extract as a food additive

Executive summary

The purpose of Application A1129 is to seek an amendment to the Australia New Zealand Food Standards Code (the Code) to permit monk fruit extract, an intense sweetener, to be used as a food additive. FSANZ has not previously assessed the safety of monk fruit extract.The submitted data are considered adequate to define the hazard of monk fruit extract.

Monk fruit extract is derived from the fruit of *Siraitia grosvenorii*, a perennial vine native to southern China. The sweet components of monk fruit extract are cucurbitane triterpene glycosides known collectively as mogrosides. The predominant component of commercial monk fruit extracts is mogroside V, which typically represents 30 to 40% of the extract.

The available evidence presented to support the proposed uses of monk fruit extract, namely, as an intense sweetener in table-top sweeteners and other ready-to-consume foods, is clearly articulated in the application. The evidence provides adequate assurance that the food additive, in the proposed form and usage levels, is technologically justified and has been demonstrated to be effective in achieving its stated purpose of intense sweetener.

Metabolism studies indicate that mogroside V is largely degraded in the intestinal lumen, with numerous metabolites formed. A number of the metabolites can be measured in plasma, urine, liver and other organs, indicating systemic absorption, but there is also excretion of parent compound and metabolites in the faeces, which suggests that systemic absorption is only partial.

The acute toxicity in mice could not be established because the toxicity of monk fruit extract is very low. Repeat-dose subchronic studies showed no adverse effects from monk fruit extract at the highest doses tested which were 5 g/kg bw/day in mice, 7.07 g/kg bw/day in male rats, 7.48 g/kg bw/day in female rats, and 3 g/kg bw/d in dogs.

A reproductive and developmental screening study of monk fruit extract containing 30% mogroside V w/w, found no adverse clinical or reproductive effects on male or female rats of the P generation, or on F1 pups up to postnatal day 13, of daily doses of monk fruit extract to the P generation up to 4000 mg/kg bw/day. Treatment did not have any demonstrable effects on development or on markers of sexual differentiation or thyroid function in the F1 pups. No chronic toxicity/carcinogenicity studies are available, but because monk fruit extract is not

genotoxic and no lesions that might progress to neoplasia by nongenotoxic mechanisms were observed in subchronic studies, such studies are not considered to be necessary.

Monk fruit is a traditional food and folk medicine in China, and monk fruit extract has a long history of use in Japan. Furthermore monk fruit extract has been available in the USA for a number of years and was recently approved in Canada. No adverse effects on human health or development associated with monk fruit extract consumption has been reported in the populations of any of those countries. There is no evidence from human studies that there are any adverse effects of monk fruit consumption, although the studies were not designed as tolerance studies.

Based on the reviewed toxicological data, it is concluded that in the absence of any identifiable hazard, an Acceptable Daily Intake (ADI) ‘not specified’ is appropriate for monk fruit extract. A dietary exposure assessment is therefore not required.

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1 Introduction

Monk fruit extract is derived from the fruit of *Siraitia grosvenorii,* a perennial vine native to southern China, and a member of the Cucurbitaceae family. Previous scientific names of this plant are *Momordica grosvenorii* and *Thaldiantha grosvenorii*. Alternative common names of the fruit include luo han guo, longevity fruit, the Buddha's fruit and arhat fruit.

The monk fruit itself has been used whole or in dried powder form for many centuries in China in beverages and traditional medicines. Monk fruit extract became recognised for its potential use as an intense sweetener in the mid-1970s, where water and ethanol extraction of the principle component of the monk fruit resulted in an intensely sweet substance. Monk fruit extract-containing food products are already permitted in a number of countries overseas, including in the US since 2007.

The components of the monk fruit extract that impart the sweetness are collectively known as mogrosides (cucurbitane triterpene glycosides). Pure mogroside V is the primary component, exhibiting a sweetness of between 250 and 400 times that of sucrose. Monk fruit extract is claimed to have a number of advantages over other already approved intense sweeteners. In particular, it has a relative lack of bitter taste compared with certain other intense sweeteners, for example, saccharin and acesulfame K. It can be used as a sugar substitute in baking (as it has high temperature stability and no unpleasant aftertaste).

1.1 Scope of the current hazard assessment

The application is to seek an amendment to the Code to permit monk fruit extract, an intense sweetener, to be used as a food additive. FSANZ has not previously assessed the safety of monk fruit extract. The aims of the current hazard assessment are to:

- Evaluate the technological purpose of monk fruit extract
- Review the available data on the toxicology of monk fruit extract to determine its safety as a food additive and
- If appropriate, establish a health-based guidance value for monk fruit extract.

2 Food technology assessment

2.1 Characterisation of the food additive

2.1.1 Identity of the food additive

The primary component of monk fruit extract is mogroside V. Identification details for mogroside V taken from the application have been verified against the monograph for monk fruit extract in the United States (US) Pharmacopeial Convention (2016) Food Chemicals Codex (10th edition).

Generic common name:	Monk fruit extract
Molecular formula:	$C_{60}H_{102}O_{29}$ (mogroside V)
Accepted IUPAC[1] systematic name:	(2R,3R,4S,5S,6R)-2-[[(2R,3S,4S,5R,6R)-6-[[(3S,8S,9R,10R,11R,13R,14S,17R)-17-[(2R,5R)-5-[(2S,3R,4S,5S,6R)-4,5-dihydroxy-3-[(2R,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy-6-[[(2R,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxymethyl]oxan-2-yl]oxy-6-hydroxy-6-methylheptan-2-yl]-11-hydroxy-4,4,9,13,14-pentamethyl-2,3,7,8,10,11,12,15,16,17-decahydro-1H-cyclopenta[a]phenanthren-3-yl]oxy]-3,4,5-trihydroxyoxan-2-yl]methoxy]-6-(hydroxymethyl)oxane-3,4,5-triol
Other name:	Mogrol-3-O-(β-D-glucopyranosyl(1→6)-β-D-glucopyranoside)-24-O-((β-glucopyranosyl (1→2))-(β-D-glucopyranosyl(1→6))-β-D-glucopyranoside)
CAS[2] number:	Mogroside V [88901-36-4]
Commercial names:	Mogroside V; Monk fruit extract; Luo han guo extract
Product brand name:	PureLo® (BioVittoria Ltd)

2.1.2 Physical and chemical properties of the food additive

The commercial preparation of monk fruit extract has been concentrated to optimise the concentration of mogroside V. It typically comprises a concentration of mogroside V of 30-40%. However there are preparations with a concentration of up to 90% mogroside V available (US FDA 2015). Other components of the commercial preparation include 11-oxomogroside V (1-10%), siamenoside I (1-10%), mogroside IV (1-10%), water (1-6%) and ash (0-2%). The balance is made up of protein fragments.

Commercial monk fruit extract is a powder, light yellow-brown in colour, readily soluble in water. The extract has a slight characteristic odour of the monk fruit. Other chemical

[1] International Union of Pure and Applied Chemistry
[2] Chemical Abstracts Service

properties are given in Table 1, and the structural formula of mogroside V is given at Figure 1 below.

Table 1: Chemical and physical properties of monk fruit extract

Property	Value
Formula weight (g/mol)	1286 (mogroside V)
Melting point	Approximately 310°C (depends on exact composition)
Moisture content	Less than 6%
Ash content	Less than 2%
Particle size	Typically 100-250µm

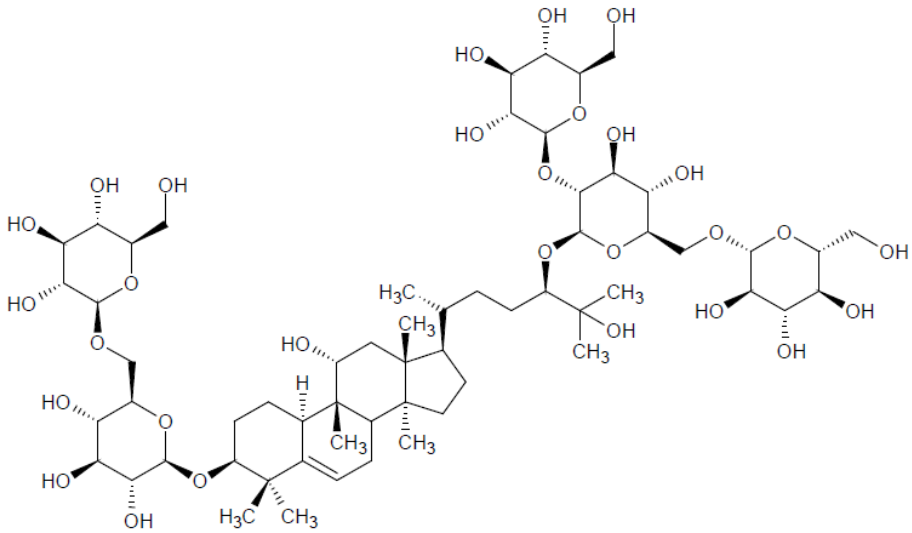

Figure 1: Structural formula of mogroside V (source: application)

2.1.3 Technological purpose of the food additive

The technological purpose that monk fruit extract fulfils is that of an intense sweetener. The Code defines intense sweetener as a substance that 'replaces the sweetness normally provided by sugars in foods without contributing significantly to their available energy'. Intense sweeteners may be added to a food only in amounts necessary to wholly or partially replace the sweetness normally provided by sugars.

Depending on the mogroside V concentration, the sweetness of commercial monk fruit extracts may be up to 200 times that of sucrose (Marone et al. 2008). The proposed concentration of use in a range of ready-to-consume foods, including fruit and vegetable spreads, confectionery and biscuits, among others, ranges from 1000 mg/kg (0.1%) to 5000 mg/kg (0.5%). The proposed concentration of use of the extract in table-top sweeteners is 8000 mg/kg (0.8%).

The specific available energy of a typical monk fruit extract has been analysed to be 16 kJ/g (Saraya Co Ltd 2006). Monk fruit extract can provide the sweetness level normally provided by sugars while only contributing around 130 J of energy per gram of food, which is 0.7% of sugar's energy value. Therefore, monk fruit extract fulfils the technological purpose of an intense sweetener.

2.1.4 Technological justification for the food additive

The technological justification for using monk fruit extract as an intense sweetener centres around its potential use as a sugar substitute in baking and its lack of bitter taste compared to other intense sweeteners.

Specifically, the application indicates that a sweetener blend containing 0.8% monk fruit extract and a sugar alcohol is useful as a sugar substitute in baking. This is because it has high temperature stability and no unpleasant aftertaste, which is typically not the case with other intense sweeteners such as aspartame or saccharin (University of Illinois 2014).

Secondly, monk fruit extract has a different sensory profile compared with other intense sweeteners. It has a relative lack of bitter taste, especially when compared with saccharin and acesulphame K (Kuhn et al. 2004). Monk fruit extract has been found to exhibit bitterness roughly equivalent or less than that exhibited by sucrose (Kim et al. 2015). Other characteristics of the sensory profile include a honey odour and flavour, and liquorice flavour. These sensory characteristics may help make monk fruit extract a more palatable and appealing choice of intense sweetener for some consumers compared with other intense sweeteners currently available on the market.

Since monk fruit extract is derived from a plant product, it may be considered a natural sweetener; this aspect may also be appealing to certain food manufacturers and consumers.

FSANZ is satisfied that the applicant has provided sufficient evidence supporting the technological justification for use of monk fruit extract as an intense sweetener food additive.

2.2 Analytical method for detection of the food additive

The applicant has provided information regarding the detection and quantification of mogroside V in a food matrix, performed using high-performance liquid chromatography with UV detector (HPLC-UV) with an ODS[3] column, which is comparable to the method described in the US Pharmacopeial Convention (2016) Food Chemicals Codex (10th edition). Quantification of the amount of mogroside V present in the sample is calculated by comparing the chromatogram peak areas of the sample and of a standard of known concentration. Pure mogroside V standards for analytical use are available from the US Pharmacopeial Convention (catalog number 1445448).

2.3 Manufacturing process

2.3.1 Production of monk fruit extract

Figure 2 below shows a schematic overview of the production process, with the major manufacturing being:

- Multi-stage solid-liquid extraction process to extract mogroside V from the mechanically crushed or shredded pulp of the fruit. The extract solutions from each stage are combined.

- Precipitation of protein compounds by heating to 100 ºC, which are then removed by centrifugation, resulting in a clear solution.

[3] Octa Decyl Silane. An ODS column is filled with a packing of octadecylsilyl groups (ODS groups or C18 groups) chemically bonded to a silica gel carrier. ODS columns are used for reverse-phase chromatography.

- Filtration to remove pectin.

- Solid-phase extraction, involving:
    - Adsorption of mogrosides onto a food-grade copolymer resin column. Unwanted compounds including salts and sugars pass through the column and are disposed of.
    - Desorption of the target compounds from the column using a food-grade 60% ethanol solution, followed by distillation to remove the ethanol.

- A second solid-phase extraction using a copolymer resin column, adsorbing any unwanted non-triterpene glycosides and allowing the desired triterpene glycosides to pass through the column. This also decolours the solution.

- Vacuum concentration of the solution to approximately 20% solid content. This also removes most of the remaining ethanol.

- Spray drying at 120ºC to form a dry powder and remove any final traces of ethanol and most of the water content.

The applicant has advised that good manufacturing practices (GMP) are employed to ensure a food-safe final product.

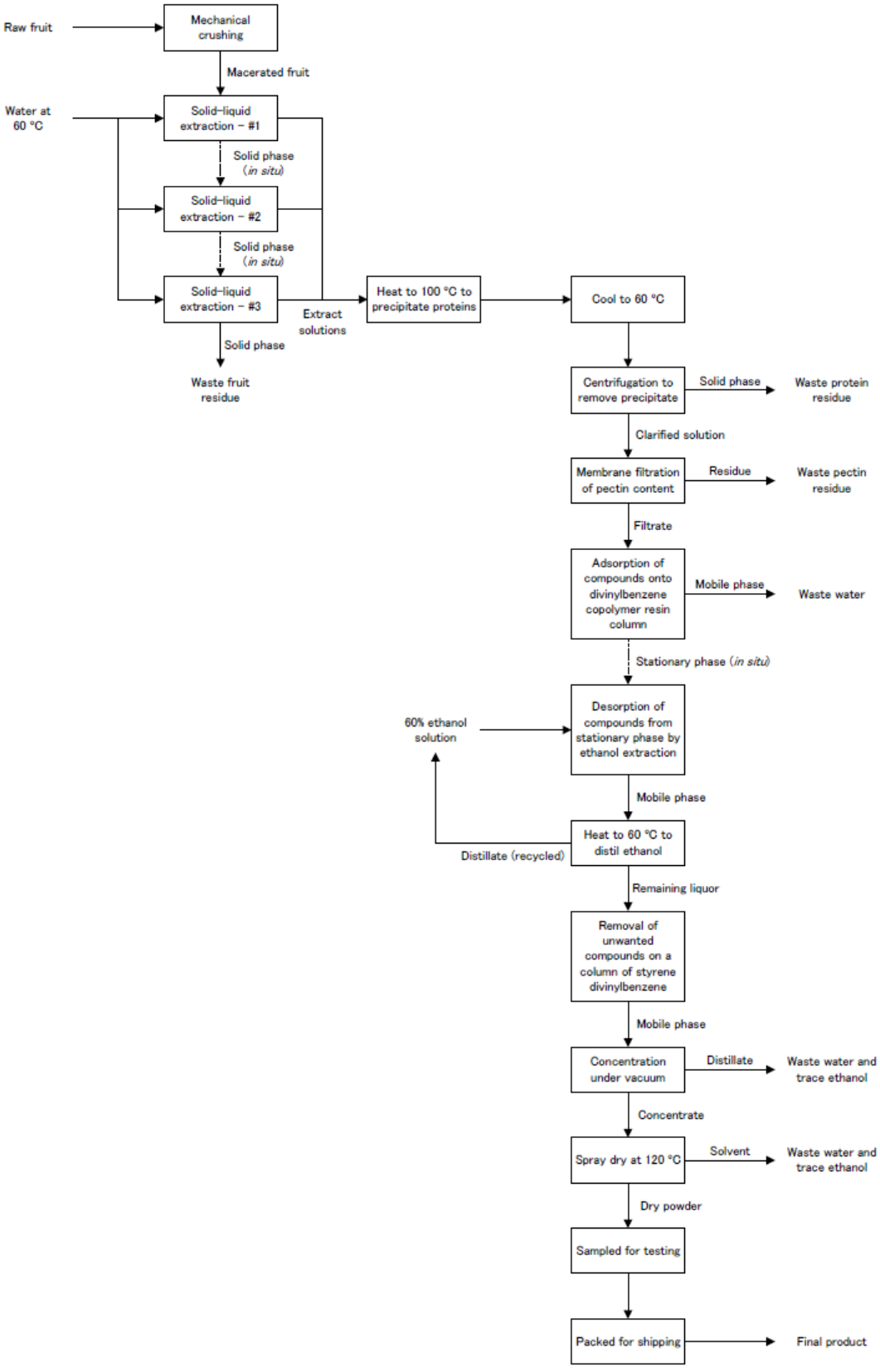

Figure 2: Flow diagram of monk fruit extract production (source: application)

2.3.2 Product specifications

For the purposes of purity specifications, the applicant has referred to the monograph for monk fruit extract in the US Pharmacopeial Convention (2016) Food Chemicals Codex (10th edition). This is listed as a primary reference for specifications in Schedule 3 of the Code. Schedule 3 also includes specifications for heavy metals if they are not included in the Code's primary or secondary references.

Table 2 provides analytical results performed on five different batches of monk fruit extract from two different manufacturers. Not all batches were tested for all analytes. These results are compared with the specifications detailed in Food Chemicals Codex and those in the Code (as applicable).The applicant advises that results presented are representative of typical analysis results.

Based on the above product specifications, monk fruit extract exceeds the Food Chemicals Codex arsenic limits for two samples, however meets the Code specifications for arsenic and all other metal contaminants.

An international standard (including specifications) for monk fruit extract has not been established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Monk fruit extract was assigned a high priority for evaluation at the 46th session of the Codex Committee on Contaminants in Food (CCFA) (March 2014), and was included on the priority list of substances proposed for evaluation by JECFA. It was to be considered at the 80th meeting of JECFA (2015). However it was removed from the agenda as no data were submitted to allow consideration. As such, there are no JECFA specifications to enable comparison.

Table 2: Comparison of Food Chemicals Codex and the Code specifications for monk fruit extract

Analysis		*Specifications for monk fruit extract*	
	Batch results from different manufacturers	**Food Chemicals Codex**	***Australia New Zealand Food Standards Code* (section S3—4)**
Lead (mg/kg)	0.12, 0.17, <1.0, <1.0, ≤1.0	<1.0	≤2
Arsenic (mg/kg)	0.03, 0.03, <1.0, <1.0, ≤0.5	<0.5	≤1
Mercury (mg/kg)	ND, ND, <0.01	N/A	≤1
Cadmium (mg/kg)	<0.5, <0.5, ≤0.1	<0.5	≤1

2.3.3 Stability

Results of stability analyses provided by the applicant indicate that the sweetness components of monk fruit extract are not significantly affected by heat treatment at 120°C as detailed in Table 3 below. The thermal stability of mogroside V in a table-top sweetener product (containing 0.8% monk fruit extract and 99.2% sugar alcohol) was also tested, and results indicated the product was stable when incubated at 120°C up to 6 hours.

Table 3: Thermal stability of monk fruit extract

	Mogroside V (%) detected	
Time/Temperature (°C)	90°C	120°C
2 hours	99.9 ± 1.2	95.1 ± 1.5
6 hours	96.5 ± 1.6	91.5 ± 1.9
24 hours	93.2 ± 1.2	86.0 ± 1.0

Stability of mogroside V was also evaluated in monk fruit extract and a table-top sweetener at temperatures of below 0°C. Mogroside V was found to not be significantly affected in either product after one month of storage at -5°C.

The effects of long term storage at room temperature on the level of mogroside V in both monk fruit concentrate and a table-top sweetener were also determined. The specification for mogroside V in monk fruit extract, as detailed in Food Chemicals Codex, is not less than 30%. Three samples of monk fruit extract stored at room temperature for approximately one, two and three years were all found to contain more than 30% mogroside V, indicating that it is stable for long durations at ambient temperature.

The theoretical content of mogroside V in a table-top sweetener is expected to be higher than 900 ppm. Four samples of a particular brand of table-top sweetener stored at ambient temperature for various lengths of time were all found to contain more than 900 ppm mogroside V, indicating good stability of mogroside V in the table-top sweetener as well.

2.4 Conclusion

The stated purpose of monk fruit extract, namely, for use as an intense sweetener in table-top sweeteners and other ready-to-consume foods, is clearly articulated in the application. Monk fruit extract exhibits a number of benefits over other intense sweeteners, as it has a relative lack of bitter taste, and performs well in baking, with high temperature stability and no unpleasant aftertaste. The evidence presented to support the proposed uses provides adequate assurance that the food additive, in the proposed form and usage levels, is technologically justified and has been demonstrated to be effective in achieving its stated purpose. Typical analysis results of the food additive indicates that it meets international purity specifications, with the exception of two analysed levels of arsenic that are greater than the Food Chemicals Codex specification, but which meet the Code specifications for this metal contaminant. The applicant will need to ensure that the final preparation meets all specifications, including the arsenic limits set in Food Chemicals Codex.

3 Hazard assessment

3.1 Background

Monk fruit extract is derived from the fruit of *Siraitia grosvenorii*, a perennial vine, native to southern China, of the Cucurbitaceae family. Former Linnaean names of this plant are *Momordica grosvenorii* and *Thaldiantha grosvenorii*. Alternative common names of the fruit include longevity fruit, the Buddha's fruit and arhat fruit.

3.1.1 Chemistry

Details of the physicochemical properties of monk fruit extract are presented in Section 2 of this report. Briefly, the sweet components of monk fruit extract are cucurbitane triterpene

glycosides known collectively as mogrosides. The predominant component of commercial monk fruit extracts is mogroside V, which typically represents 30 to 40% of the extract. Other components include 11-oxomogroside V (1-10%), siamenoside I (1-10%), mogroside IV (1-10%), water (1-6%) and ash (0-2%). The balance is made up of protein fragments.

Commercial monk fruit extract is a powder, light yellow-brown in colour, that is readily soluble in water.

3.1.2 Evaluation of the submitted data

FSANZ has assessed the submitted evidence, as well as information from other sources, concerning the safety of monk fruit extract. This includes information on history of safe use, genotoxicity, acute and subchronic toxicity in laboratory animals, and information on human tolerance and traditional use. The available data are considered suitable to assess the hazard of monk fruit extract.

3.2 Toxicokinetics and metabolism

Study of metabolism of mogroside V in the rat (Murata et al. 2010) Non-GLP

A glycoside powder (SG-gly), comprising 72% w/w mogroside V, was prepared from monk fruit. An unspecified number of Wistar rats, 9 weeks old at time of purchase, were acclimated for one week to standard laboratory husbandry conditions. They were fasted for 16 hours and then gavaged with 1 mL of a solution containing 117 mg SG-gly/mL in an unspecified vehicle, probably water. Some of the rats were killed 120 minutes after administration of SG-gly for collection of portal blood and small intestinal contents. Faeces was collected from other rats 24 hours after administration of SG-gly. Although results of urinary analysis are also reported, it is not stated in the described method when or how urine was collected.

Analysis of the small intestinal contents showed that after 120 minutes, mogroside V was partially degraded with the predominant metabolites being siamenoside I, mogroside IV, mogroside III. There were also small quantities of other mogrosides.

Free triterpenoids from monk fruit were not detected in portal blood plasma. When the plasma was treated with β-glucuronidase and sulfatase, small amounts mogroside IE and mogrol were detected. The authors suggested that mogroside IE and mogrol are conjugated in the small intestine.

The predominant metabolites found in the faeces were mogrol, mogroside IIA, and mogroside IE, although several other monk fruit triterpenoids were also present. The total amount of mogroside in the faeces was approximately 61% of the administered dose. No monk fruit triterpenoids were detected in the urine, in free or conjugated form. Based on the very limited quantities of metabolites (mogroside IE and mogrol) in portal blood and the absence of metabolites in urine, it was concluded that most of an oral dose of mogroside V is excreted without systemic absorption. The amount of mogroside IIE in the small intestine was approximately equivalent to the amount in the faeces, while the metabolite mogroside IIA was found only in the faeces. It was concluded that mogroside IIE is formed in the small intestine, and mogroside IIA in the large intestine.

The authors concluded that SG-gly was mostly degraded in the intestinal lumen with little absorption of the resulting metabolites, which were largely excreted directly in the faeces. However, the authors also noted that a number of pharmacological effects of monk fruit glycosides have been reported in animal models, which implies at least some systemic absorption. They suggested that mogrol may be the pharmacologically active metabolite. At 2 h after a single oral administration, concentration of mogrol in portal blood was 0.36 ± 0.22

nmol/mL, whereas over 24 h after administration, faecal excretion of mogrol was 21.34 µmol.

In vitro and in vivo investigations of metabolism and distribution of mogroside V (Xu et al 2015). Non-GLP

In vitro experiments reported in this paper were incubation of mogroside V with human intestinal bacteria and incubation with S9 fraction prepared from rat liver. The *in vivo* experiment was a 10-day study conducted in rats. The test article in all the experiments was mogroside V, purity >98%. Metabolites were detected using HPLC in tandem with ESI ion trap (IT) TOF multistage mass spectrometry (HPLC-ESI-IT-TOF-MS).

To determine the in vitro metabolism of mogroside V with human intestinal bacteria, fresh faeces from a healthy Chinese man were suspended in general anaerobic medium (GAM) and incubated to obtain a suspension of activated intestinal bacterial flora. Replicate tests were conducted, although the number of replicates in unclear. The incubation system for the test group included GAM, mogroside V and intestinal flora. This was compared to control groups in which either the mogroside V or the intestinal flora were omitted. All samples were incubated anaerobically for 48 h at 37°C. Fourteen metabolites were identified, including isomers of mogrosides VI, V, IV, II and monoglycosylated mogrol.

The S9 fraction from rat liver was obtained from six rats adjusted to 20 mg protein/mL in Tris-HCl solution and cofactors (KCL, $MgCl_2$, glucose 6 phosphate, NADP) added to produce the incubation system. Metabolism of mogroside V in this system was compared to control systems in which either the mogroside V or the S9 fraction was omitted but all other parts of the system were the same. Each system was incubated with agitation for 3 h at 37°C. Four metabolites of mogroside V were detected in the test group sample, all mogroside VI isomers.

For the *in vivo* part of the investigation, young (230 g ± 20 g) male Sprague-Dawley rats were assigned to two groups of four rats each, and kept in metabolism cages with *ad libitum* food and water. The total length of the in-life phase was 10 days, of which the first three days were used as an adaption period. Pre-dosing samples of faeces and urine were collected on days 4 and 5. On days 6 to 10 inclusive, the rats in the treatment group were gavaged once daily with either 50 mg/kg bw/day mogroside V in water while the rats in the control group were gavaged with an equivalent volume of physiological saline. Urine and faeces were collected throughout the experiment. Rats were killed on Day 10 with collection of blood from the heart. Heart, liver, spleen, lungs, kidneys, stomach and small intestine were collected from each rat, rinsed, weighed and stored frozen until analysed.

Fifty-eight metabolites were detected in rat faeces. Mogroside V and 29 metabolites were detected in rat urine, and 14 metabolites were detected in plasma. Mogroside and 33 metabolites were detected in liver, and the numbers of metabolites detected in spleen, lung, kidney, stomach and small intestine were 39, 39, 42, 45 and 51 respectively. Overall, 52 of the 77 metabolites were oxidation products formed by hydroxylation and/or dehydrogenation. The metabolic reactions of mogroside V also include deglycosylation, isomerization, glucosylation, and methylation.

3.3 Toxicity studies

3.3.1 Genotoxicity studies

Forward mutation assay of monk fruit extract (Hussain et al 1990) Non-GLP

This report is brief and lacks full details of the experimental method. The monk fruit extract was prepared by milling the plant material and extracting it into 80% methanol. The extract

was dried and processed into residues soluble in petroleum ether, ethyl acetate, butanol or aqueous methanol. Mutagenicity testing was performed on the original methanol extract and on the other extracts. The forward mutation assay was conducted using *Salmonella typhimurium* strain TM 677, with and without S9 fraction for metabolic activation. All the plant extracts were dissolved in DMSO and evaluated at final concentrations of 0.31, 0.62, 1.25, 2.5 and 5.0 mg/mL. Data and full experimental details are not provided, but Hussain et al (1990) reported that the plant extracts were not mutagenic or bacteriocidal.

Reverse mutation assay of monk fruit extract (Matsushima 1999) Non-GLP

The test material for this experiment was a commercial monk fruit extract. There are no details on the extraction procedure, composition or purity, but the test material was water-soluble and the negative control material, and vehicle, was distilled water. The bacterial strains used were *Salmonella typhimurium* TA98, TA100, TA1535 and TA1537, and *Escherichia coli* WP2uvrA/pKM101. The positive control articles in the assays without S9 mix were 2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide for use with TA100, TA98 and WP2uvrA/pKM101; sodium azide for use with TA1535; and 9-aminoacridine for use with TA1537. The positive control article for assays in the presence of S9 mix was 2-aminoanthracene for all the bacterial strains. The assays were conducted using the preincubation method. The dose-setting trials were conducted using concentrations of 0, 313, 625, 1250, 2500 and 5000 µg/plate, and the same doses were used for the definitive study.

No increase in revertant colonies was observed with any dose of monk fruit extract at any concentration with any of the bacterial strains, with or without the presence of S9 mix for metabolic activation. All the positive control substances produced the expected marked increase in the number of revertant colonies. It was concluded that monk fruit extract did not have a mutagenic effect on the test microorganisms.

Mouse bone marrow micronucleus test of monk fruit extract (Shirasu 1990) Non-GLP

The test material in this assay was a water-soluble commercial extract of monk fruit, but details of composition are not provided. The test subjects were ddY-type male mice, that were seven weeks at the time of study start. Mice were group-housed in cages under standard conditions of controlled temperature and humidity. Food and water were provided *ad libitum*. Six mice/group were gavaged with 0, 500, 1000 or 2000 mg/kg bw, twice at 24 hour intervals. A further six mice were dosed with 10 mg/kg bw mitomycin C, also administered by gavage twice at 24 hour intervals, as a positive control group. The dose volume for the negative control and monk fruit extract groups was 20 mL/kg bw, while the dose volume for the positive control group was 10 mL/kg. Mice were killed 24 hours after the last dose. Bone marrow was collected from femurs and processed to slides for counting of micronuclei-containing polychromosomal erythrocytes (MNPCEs) per 1000 polychromosomal erythrocytes (PCEs).

All mice survived to scheduled termination. The frequency of MNPCE/1000 PCE was not significantly increased in mice treated with monk fruit extract, when compared to negative control mice. The frequency of MNPCE was significantly increased in the positive control group, confirming the validity of the assay. It was concluded that monk fruit extract did not induce micronuclei in bone marrow cells.

Mouse bone marrow micronucleus test of flavonoids isolated from Siraitia grosvenorii *(Yang et al 2015) Non-GLP*

This study was a combined subchronic toxicity study and micronucleus test. Flavonoids were extracted from *Siraitia grosvenorii* by pulsed electric field technology. The subject animals

were male ICR mice with a mean bodyweight of 20 g. Details of housing and environmental conditions were not reported. Mice, 10/group, were treated by daily oral gavage, at a dose volume of 0.4 mL, with 0, 1.25, 2.50 or 5.0 g/kg bw/d flavonoids for 40 days. A further 10 mice were treated with 40 mg/kg bw cyclophosphamide as the positive control, although the frequency and timing of the administration of cyclophosphamide is not clear from the report. The negative control material, and vehicle for the flavonoid administration, was distilled water. Mice were subject to daily clinical observations, and food consumption and bodyweight were determined at 4-day intervals. Mice had *ad libitum* access to food and water. Mice were killed by dislocation of the cervical spine after 40 days on study, and were subject to gross necropsy and recording of weights of selected organs. Bone marrow collected from the sternum was used to measure the micronucleus rate of 1000 polychromatic erythrocyte (PCE) cells (MNPCE/1000 PCE).

All mice survived to scheduled termination, and there were no treatment-related effects on clinical observations, food consumption, bodyweight changes, gross lesions at necropsy, or organ:bodyweight ratios of heart, liver, spleen or kidneys. The positive control group showed a significant increase in micronuclei in PCE cells, confirming the validity of the assay. The groups treated with flavonoids, in contrast, had lower incidence of micronuclei than the negative control group that was treated with distilled water, and this decrease showed a dose-response relationship. Overall, the results showed that flavonoids extracted from *Siraitia grosvenorii* had no genotoxic effect on chromosomes of mice, and may reduce chromosomal damage in mice.

A study published in Chinese by Su et al (2005) reported negative results for 'mogrosides' in a bacterial reverse mutation assay (Ames test) using *Salmonella typhimurium* strains TA97, TA98, TA100 and TA102, but only the abstract of the paper is available in English. Jin et al (2007) cite unpublished reports of assays contracted by the Ministry of Health, Labour and Welfare of Japan, which showed that monk fruit extract was negative in an *in vitro* chromosome aberration assay, a bacterial reverse mutation assay (Ames test) and an *in vivo* micronucleus test.

3.3.2 Studies in experimental animals

3.3.2.1 Acute toxicity studies

Lee (1975) reported that the LD_{50} in mice of a lyophilized extract of monk fruit was in excess of 10 g/kg bw, but experimental details were not given.

Acute toxicity of water-souble monk fruit extract in mice (Shirasu 1990) Non-GLP

The test material in this assay was a water-soluble commercial extract of monk fruit, but details of composition are not provided. The test subjects were ddY-type male mice. They were 6 weeks old at the time of purchase and were acclimated to laboratory conditions for one week prior to study start. Mice were group-housed (6/cage) under controlled conditions of temperature and humidity. Food and water were provided *ad libitum*. Mice were assigned to groups of 3/group, and gavaged twice over 24 hours with doses of 10, 100, 1000 and 2000 mg/kg bw. All mice were observed for 4 days after the second dose administration. All mice survived the study and there were no treatment-related clinical signs. The authors concluded that the LD_{50} could not be calculated from these results. It is apparent from these results that the LD*50* was in excess of 2 g/kg bw.

Acute toxicity study of methanol:water (80:20) extracts of monk fruit in mice (Hussain et al 1990) Non-GLP

The acute toxicity of various extracts of monk fruit were reported by Hussain et al (1990)

although details of the experimental method are limited. The monk fruit extract was prepared by milling the plant material and extracting it into 80% methanol. The extract was dried and processed into residues soluble in petroleum ether, ethyl acetate, butanol or aqueous methanol. Acute toxicity was assessed in male Swiss-Webster mice between the ages of 4 and 6 weeks. Test material was administered by oral gavage as a single dose of 0, 1 or 2 g/kg bw in 1% aqueous carboxymethylcellulose. Mice were observed for 14 days after dose administration, and bodyweights were measured over the same period, at unspecified intervals. None of the extracts was acutely toxic at ≤ 2 g/kg bw.

3.3.2.2 Subchronic toxicity studies

Thirteen-week dietary toxicity study of monk fruit extract in rats (Jin et al 2007) Non-GLP

The test material for this study was an extract of *Siraitia grosvenori*, although there is a lack of information on the extraction procedure or the composition of the extract. The test subjects were Wistar Hannover (GALAS) rats. Male and female rats were obtained at 4 weeks of age and acclimated for one week prior to study start. They were group-housed, three/cage under conventional laboratory environmental conditions. Water and basal powdered diet were provided *ad libitum*. Rats were assigned, 8/sex/group, to dose groups that were administered 0, 0.04%, 0.2%, 1% or 5% w/w of powdered monk fruit extract in the diet for 13 weeks. During the in-life phase, rats were subject to daily clinical observations, and weekly determination of bodyweight, estimated food consumption and estimated water consumption. At the end of the in-life phase, rats were fasted for 16 hours, and blood samples for haematology and clinical chemistry were collected under anaesthesia before the rats were exsanguinated and necropsied. Fresh organ weights of brain, heart, lungs, liver, kidneys, spleen, thymus, adrenal glands, pituitary gland, thyroid glands, testes, uterus and ovaries were recorded and preserved for histopathology. Other organs preserved for histopathology were artery, bone with marrow, coagulating gland, oesophagus, epididymides, large intestine, lymph node, mammary gland, pancreas, peripheral nerve, prostate gland, salivary gland, skeletal muscle, skin, small intestine, spinal cord, stomach, urinary bladder, tongue, trachea, vagina, eyeballs, Harderian glands and testes. Preserved tissues from the control and 5% groups were processed to slides for histopathological examination.

All the rats survived to scheduled termination and there were no treatment-related clinical observations, or treatment-related effects on bodyweight, bodyweight gain, food consumption or water intake. Statistically significant differences in group mean haematology parameters between control males and treated males were limited to numerically slight increases in the group mean values for band neutrophil ('stab cell') and monocyte counts in males treated with ≥1% w/w monk fruit extract, as compared to control males. These findings were not present in females and had no histological correlates. Statistically significant differences in group mean clinical biochemistry data, as compared to sex-matched controls, were found only in females in the 5% w/w group and were a moderate increase in total cholesterol and a slight decrease in serum inorganic phosphate. These differences were not present in males and had no histological correlates.

The group mean liver:bodyweight ratio of males in the 5% w/w group was slightly higher than that of the control male group, and this difference reached statistical significance, but there was no corresponding effect apparent in females and no histological correlate. Group mean absolute and relative pituitary weight showed a slight but statistically significant increase in 5% w/w females when compared to female controls, but there was no corresponding change in males and no histological correlate.

In conclusion, the NOAEL for monk fruit extract in this study was 5% w/w in the diet, which corresponded to an average daily intake of 2.52 g/kg/day in males and 3.2 g/kg bw/day in females.

Twenty-eight day dietary study of monk fruit concentrate in rats (Marone et al 2008)
Regulatory status: GLP; conducted in compliance with OECD and US FDA guidelines.

The test material was a commercial concentrate from monk fruit with the trade name PureLo®. PureLo® is comprised of mogrosides (62%), with the other components including protein and melanoidins. Mogroside V is estimated to make up 39% of the total mass of PureLo®.

Sprague-Dawley rats, 7 weeks old at study start, were assigned to four groups of 20 rats/sex/group. Rats were individually housed under standard laboratory environmental conditions, and provided with water *ad libitum*. Rats were fed a standard laboratory rat chow to which the test article and sucrose were added to reach target doses while maintaining equal quality of nutrient content and closely similar calorie content. The test diets contained 0, 10000, 30000 or 100000 ppm, and were fed *ad libitum*.

Parameters measured during the in-life phase were twice daily cageside observations, weekly detailed clinical observations, weekly bodyweights, weekly food consumption, and opthalmological examination prestudy and on study day 23. Urine and blood were collected near the scheduled termination of the in-life phase for urinalysis, haematology and clinical chemistry. Rats were exsanguinated under anaesthesia on Day 29 for males and Day 30 for females, and subject to gross necropsy. Fresh weights of liver, kidneys, adrenals, brain, heart, thymus, spleen, ovaries, testes, epididymides and uterus, as sex-appropriate, were recorded. A comprehensive range of tissues and organs was preserved for histopathology, and histopathological examinations were conducted on all these tissues and organs for the control and 100000 ppm groups.

All rats survived to scheduled termination and there were no adverse clinical observations. There were no abnormal ophthalmological findings in any rat. Bodyweights and bodyweight gains of the 10000 and 30000 ppm groups were comparable to those of controls. Group mean bodyweights of rats were significantly lower than those of sex-matched controls for males on Days 6 and 13, and females on Days 13, 20 and 26. Group mean bodyweight gain over the course of the study was 85% that of sex-matched controls for 100000 ppm males and 70% that of female controls for 100000 ppm females, a statistically significant difference in both sexes. There were some significant differences in group mean food consumption of the 100000 ppm rats compared to values of sex-matched controls. Group mean food consumption in the interval Day 0-6 for 100000 ppm males was 85% that of controls, although it was not significantly different over the entire course of the study. In the intervals Days 13-20, it was 92% that of controls in females, and over the entire in-life phase, Days 0-26, it was 92% that of controls in females. Group mean daily feed efficiency was only significantly different to that of sex-matched controls for 100000 ppm males in the interval Day 0-6, when it was 83% that of controls.

There were few statistically significant differences in group mean values for haematology, and none was considered adverse. In 100000 ppm males, group mean haemoglobin concentration and haematocrit were slightly but significantly increased relative to those of male controls, while group mean total leukocyte count and absolute lymphocyte count were slightly but significantly lower than those of control males. However, no corresponding significant differences in any of these parameters were evident in females. Group mean prothrombin time of females treated with ≥ 30000 ppm PureLo® was slightly shorter than in female controls, but there was no corresponding effect in males. The authors commented that the statistical significance of the prothrombin time findings appeared to be driven by one moderately low value in the 100000 ppm group combined with few data points for the control group due to suboptimal samples. Although the normal laboratory ranges for the haematology data were not provided, all group mean values were close to those of the

control groups.

Group mean bilirubin concentration was significantly decreased in males consuming ≥30000 ppm PureLo® and females consuming ≥10000 ppm PureLo®, but a decrease in plasma bilirubin is not considered to be an adverse effect. Statistically significant increases in group mean total plasma protein were present in both sexes at ≥ 30000 ppm, and appeared to be driven by an increase in globulin in females, but the changes were very slight and not considered to be adverse. Females but not males in the 100000 ppm group had very slight, but statistically significant, elevation in group mean plasma potassium and decrease in group mean plasma chloride when compared to female controls, but these changes were not considered to be adverse. Urinalysis results showed no treatment-related adverse effects, and none of the differences, when compared to sex-matched controls, in group mean haematology, clinical chemistry or urinalysis values had any histological correlate.

No treatment-related lesions were found on gross necropsy. Group mean liver to bodyweight ratio was increased, relative to that of controls, in males in the 100000 ppm group and females in all treated groups. The organ-to-bodyweight ratios of males in the 100000 group were increased, relative to those of controls, for testes, adrenals and epididymides. There was no corresponding effect in the adrenals of females. The group mean ovary to bodyweight ratio was increased, relative to that of controls, in 100000 ppm females. None of these differences had any corresponding microscopic findings and they are not considered to be adverse. The authors remarked that the changes in liver weight may have been due to the protein fraction of PureLo®.

In conclusion, the NOAEL identified by this study was 100000 ppm PureLo® in the diet, corresponding to an average daily intake of 7.07 g/kg bw/day in males and 7.48 g/kg bw/day in females.

Ninety day oral gavage study of monk fruit extract in dogs (Xiaojian et al, 1996; Qin et al, 2006) Non-GLP

The test material for this study was monk fruit extract produced by the Natural Plant Product Factory, Guilin S&T New Technology Company. In the paper by Qin et al (2006) this test material is identified as PureLo®. The batch numbers of the five lots of monk fruit extract used in the study are the same in the report by Xiaojian et al (1996) and the published paper by Qin et al (2006). Test subjects were crossbred dogs, aged between 24 and 30 weeks and weighing between 8.0 and 9.0 kg. Dogs were randomized into four groups with 3 dogs/sex/group. Each group was housed in a separate room but environmental conditions of temperature, humidity, ventilation and light-dark cycle were the same in each room, and were standard laboratory husbandry settings. Dogs were housed in individual crates, provided with distilled water *ad libitum*, and fed a rice-based diet that included fish, vegetables and cooked pork. Two of the four groups were control groups, and were gavaged with distilled water at 10 mL/kg bw/day. The other two groups were gavaged daily with 3000 mg/kg bw/day of the test material, also at a dose volume of 10 mL/kg bw/day. One treatment group and one control group were scheduled to be killed on Day 29, and the other treatment and control groups were scheduled to be killed on Day 91.

During the in-life phase, clinical observations, food consumption and water intake were recorded daily, and bodyweight, blood pressure, heart rate and respiration were measured weekly. Urine was collected on Day 0 and weekly thereafter for urinalysis. Fasted blood samples were collected for haematology and clinical chemistry on the day prior to scheduled termination. At scheduled termination, the stomach and intestinal tract of each dog was examined, and fresh weights were recorded for heart, liver, lungs, kidneys and spleen. In addition, samples of heart, liver, lungs, kidney and spleen were preserved for histopathology. All dogs survived to their scheduled termination, and there were no treatment-related clinical

observations. Food consumption was comparable in all groups. The group mean bodyweight of the dogs in the treatment group for the 28 day study was slightly greater than that of their control group and this difference persisted through the study, leading to a significantly higher group mean bodyweight on Days 14, 21 and 28. However, there was no significant difference in group mean bodyweight gain, and there was no parallel difference in group mean bodyweight between treated and control dogs assigned to the 90-day study.

There were no consistent differences between group mean values for haematology, clinical chemistry or urinalysis for control and treated dogs. There were no treatment-related findings on gross necropsy or on histopathology, and there was no significant difference in group mean organ weights of heart, liver, lungs, kidney and spleen between controls and treated dogs.

In conclusion, the NOAEL for PureLo® administered to crossbred dogs for up to 90 days was 3 g/kg bw/day.

3.3.3 Other animal studies

Forty-day oral gavage study of flavonoids isolated from Siraitia grosvenorii *in mice (Yang et al 2015) Non-GLP*

Parts of this study has been described in the section on genotoxicity, because the study was a combined subchronic toxicity study and micronucleus test. Flavonoids were extracted from *Siraitia grosvenorii* by pulsed electric field technology. The subject animals were male ICR mice with a mean bodyweight of 20 g. Details of housing and environmental conditions were not reported. Mice, 10/group, were treated by daily oral gavage, at a dose volume of 0.4 mL, with 0, 1.25, 2.50 or 5.0 g/kg bw/d flavonoids for 40 days. The negative control material, and vehicle for the flavonoid administration, was distilled water. Mice were subject to daily clinical observations, and food consumption and bodyweight were determined at 4-day intervals. Mice had *ad libitum* access to food and water. Mice were killed by dislocation of the cervical spine after 40 days on study, and were subject to gross necropsy and recording of weights of selected organs.

All mice survived to scheduled termination, and there were no treatment-related effects on clinical observations, food consumption, bodyweight changes, gross lesions at necropsy, or organ:bodyweight ratios of heart, liver, spleen or kidneys. The NOAEL for flavonoids extracted from *Siraitia grosvenorii* in this study was 5 g/kg bw/day. However, the description of the study is considered to be of limited value because of the lack of information concerning the composition of the test material, and because of the limited endpoints assessed.

Acute toxicity in mice and four-week study in dogs (Su et al 2005) Regulatory status: Unknown

This paper is in Chinese, with only the abstract available in English. Consequently the validity of the study cannot be assessed. However, the authors report that mogrosides showed no acute toxicity in mice at an intragastric dose of 10 g/kg bw. The authors also reported that dogs administered 3.0 g/kg bw/day for four weeks showed no treatment-related changes in ‘blood constituents’, liver function, kidney function, blood glucose level, urine glucose level, or morphology of heart, liver, kidneys, lungs or spleen.

3.4 Reproductive and teratogenicity studies

No adverse effects of *Siraitia grosvenorii* extracts on reproductive organs have been observed in in repeat-dose studies in animals, and no evidence of adverse reproductive or

developmental effects of mogrosides, cucurbitane glycosides or triterpenoids was found in searches of the scientific literature.

Reproductive and developmental screening study of monk fruit extract in Sprague Dawley rats (ICP Firefly Pty. Ltd. 2018). Regulatory status: GLP; based on OECD Guideline 421.

The test article for this study was monk fruit extract comprising 30% mogroside V. Monk fruit extract was prepared daily as dosing solutions of 10, 20 and 40% w/w in distilled water as the vehicle. Stability of dosing solutions for ≥ 26 h was confirmed by analysis. Distilled water was also the control article. Dose analysis for mogroside V was performed on samples collected from the dosing formulations prepared on Days 1, 29 and 34. Samples did not differ from the intended concentrations by more than 6.6%, and were suitable for use on study. Stability of dosing solutions for ≥ 26 h was confirmed by repeat analysis of the 40% dosing formulation prepared on Day1.

The parent generation for this study comprised SPF Sprague Dawley rats, 52/sex, purchased at 9-10 weeks of age. Rats were randomly assigned to treatment groups, 13/sex/group. Rats were group-housed by sex, up to 5/group, through 5 days of acclimation to standard laboratory environmental conditions, and through14 days of treatment prior to mating. For mating, one male and one female from the same treatment group were housed together. Mated females were individually housed through pregnancy and housed with their pups through lactation. Water and standard laboratory rat chow were provided *ad libitum*. Females were assessed pre-treatment for normal oestrus cycles on days 1, 5, and 10.

From 14 days prior to mating, all P generation rats were gavaged once daily with 0, 1000, 2000 or 4000 mg/kg bw/day. Volumes of dose formulation were 9.77, 9.60 and 8.98 mL/kg for the 1000, 2000 and 4000 mg/kg bw/day groups respectively. The volume of vehicle administered to the control group was not stated, but it was stated that the total dose volume would not exceed 10 mL/kg bw. Dosing of female rats continued through mating and gestation. In the treated groups, 10 dams were allowed to rear their young, with preference given to those with larger litters, and these dams were dosed for 13 days after parturition. Dosing of male rats continued through mating and for 15 days after mating, for a minimum of 28 consecutive days of dosing. In the treated groups, the 10 males corresponding to the dams allowed to rear their young were selected for further collection of tissues and samples, and for statistical analysis of results.

Bodyweight and food consumption were recorded weekly throughout the pre-treatment and treatment periods. Males were weighed on receipt and weekly during the study. Female rats were weighed on receipt, weekly prior to mating, on presumed gestation days 0, 7, 14 and 20, within 24 hours of parturition and on days 4 and 13 postpartum. Clinical observations were recorded daily. Litters were examined as soon as possible after birth to determine number and sex of pups, numbers of stillborn and live pups, and presence of gross abnormalities. Pups were weighed at birth and on days 4 and 13. Anogenital distance of all pups was measured on postnatal day 4, and the number of nipples of male pups was counted on postnatal day 13. T4 was measured by cardiac puncture in 2 pups per litter on postnatal day 4, and from 2 pups per litter on postnatal day 13.

Males were weighed and killed 15 days after mating, and blood was collected for serum T4 determination. Males were subject to gross necropsy and to weighing and fixation of the testes, epididymides, prostate and seminal vesicles with coagulating glands. Dams were weighed and killed on postpartum day 13, vaginal smears were prepared, blood collected for serum T4 determination, and a full gross necropsy performed. Ovaries and uterus, including cervix, were collected. The number of implantation sites was recorded for each dam, the reproductive tract and ovaries were weighed and fixed for histopathology. Additional tissues preserved from P generation rats were adrenals, liver, kidneys and thyroid glands. Pups

were killed on postnatal day 13 and examined for gross abnormalities. The thyroid of one male and one female pup per litter was fixed for histopathology. Histopathology was performed on tissues of the 40% group and control group.

All female rats showed evidence of normal oestrus cycles. All P generation rats survived the study, there were no treatment-related effects on food consumption in the P generation, and there were no treatment-related effects on bodyweight in either the P generation or the F1 generation. Dosing with ≥2000 mg/kg bw/day caused both males and females of the P generation to pass soft, sticky faeces but this was not considered to be an adverse effect. treatment had no effect on the number of female rats that became pregnant, length of gestation, number of stillbirths, number of perinatal deaths, litter sizes, or sex ratios within litters. Pups did not exhibit any treatment-related clinical signs, the test article had no effect on anogenital distance in pups, and no male pups exhibited retention of areolae or nipples. Treatment had no effects on group mean T4 levels of P generation or F1 generation rats, as compared to sex-matched controls on the same day. T4 levels increased in all F1 groups, including controls, between postnatal day 4 and postnatal 13, indicating normal maturation of the pituitary-thyroid axis. There were no treatment-related effects on gross necropsy findings, fresh organ weights, or histopathology findings in any P generation rats, or on the number of implantation sites in P generation females.

It was concluded that under the conditions of this study, the NOAEL for monk fruit extract containing 30% mogroside V was 4000 mg/kg bw/day.

3.5 Carcinogenicity studies

There are no carcinogenicity studies available in the scientific literature. Such studies are not considered to be necessary, since the results of genotoxicity assays are negative and there is no evidence from subchronic studies of lesions that could lead to neoplasia through nongenotoxic mechanisms.

3.6 Human tolerance studies

There are few human tolerance studies of monk fruit extract available in the scientific literature. However it is noted that monk fruit has a long history of human use in China, having been cultivated for use as a drink and as medicine for hundreds of years, and was listed as a medicinal and edible species by the China Ministry of Health in 1987 (Li et al 2014). The availability of monk fruit in the USA, largely from Chinese grocery and herb stores, has been documented since the late nineteenth century (Heimbach 2009). Monk fruit extract has a long history of use in Japan (Heimbach 2009).

Two unpublished studies were cited in a GRAS exemption claim for PureLo® monk fruit extract that was submitted to the US FDA (US FDA 2010). In the first of the unpublished studies, a cross-over design was used to compare the effect of PureLo® on blood glucose level to that of sucrose. After fasting overnight, healthy people (5/sex) aged 19-25 consumed 200 mg/kg bw of PureLo® in water. Blood glucose was determined at 0, 15, 30, 60, 120 and 180 minutes after dosing. Three days later, again after fasting overnight, the same subjects consumed 3000 mg/kg bw sucrose in water, and blood glucose was determined at the same intervals. Ingestion of sucrose resulted in a 70% increase in blood glucose within 15 minutes, and blood glucose gradually returned to normal over 3 hours. However PureLo® had no effect on blood glucose. No adverse effects of PureLo® consumption were reported. In the second unpublished study, a similar cross-over design was used. The subjects were six healthy men aged 19-25 years. After an overnight fast, they consumed 200 mg/kg bw of PureLo® in water, and blood samples were collected at 0,1,2,3, and 6 hours after consumption. Three days later the experiment was repeated, they consumed water instead, and blood samples were taken according to the same schedule. Blood was analysed for

activities of ALP, GGT, ALT, AST and LDH as markers of liver function. There was no statistically significant effect of consumption of PureLo® on the activities of the liver enzymes, when compared to water.

Acceptance of chocolate milk sweetened with monk fruit (Li et al 2015) Non-GLP

This is a consumer acceptance study rather than a human tolerance study. A chocolate milk made from skim milk powder was used to prepare different chocolate milks sweetened with sucrose, stevia, monk fruit extract, sucrose/stevia blends, or sucrose/monk fruit blends. The maximum amount of monk fruit extract used was 350 mg/L, and the chocolate milks were given to both adults (19 to 35 years) and children (5 to 13 years). The full details of the study design and results are not relevant to this hazard assessment, but it is noted that no adverse effects of consuming chocolate milks partially or wholly sweetened with monk fruit extract were reported.

Effects of monk fruit and other sweeteners on postprandial glucose, insulin and energy intake in male volunteers (Tey et al 2017) Non-GLP

For this randomised crossover study, 34 healthy male volunteers aged between 21 and 50, with a normal body mass index, were recruited. Exclusion criteria included major chronic disease, allergy or intolerance towards any of the test articles, and taking any drug known to affect appetite, glucose or energy metabolism. Volunteers who were dieting or whose body weight had changed by more than 5 kg in the previous 12 months were also excluded. The test beverages were 500 mL beverages containing either 0.44 g aspartame, 0.63 g monk fruit extract (50% mogroside V), 0.33 g stevia (rebaudioside A), or 65 g sucrose. Pilot testing established that these formulations were similar in their sweetness intensity, flavour and palatability, and the beverages were also formulated with strawberry extract and pink colouring to mask any potential differences. Standardised breakfasts and lunches were also used in the study.

All volunteers were asked to attend five sessions; a screening session and four test sessions. At the screening session, basic anthropometric measurements, body composition, blood pressure and fasting blood glucose were collected after overnight fasting of at least 10 hours, and participants were asked to complete a questionnaire on eating behaviour. Before each test day participants were asked to refrain from vigorous physical activity, consume an evening meal of similar composition and quantity as they consumed the evening before the other three test sessions, and fast for at least 10 hours overnight. Participants were randomly assigned to the order in which they consumed the test articles, with the orders balanced, and were blinded to the treatment allocation. On each test day, a participant would consume the pre-packaged standard breakfast between 0800 and 0900 hours. Between 1100 and 1200 hours the participant would arrive at the research centre for consumption of a fixed volume of the test beverage as a preload, followed by collection of a baseline blood sample, and appetite would be recorded. The participant would then consume one of the test beverages within 15 minutes. An hour later, the participant would be allowed to consume an *ad libitum* serving of fried rice for lunch. A series of blood samples would be taken at fixed intervals, 15, 30, 45, 60, 90, 120, 150 and 180 min after consumption of the test beverage, participants' perceptions of appetite, thirst, happiness, clear headedness and alertness were also recorded. Participants also kept a diet record and to take photos of all food and drinks consumed after they left the study site. Participants were required to have a hiatus of at least 5 days between test days. Blood was analysed for glucose and insulin levels.
Thirty participants completed the study. Participants found monk fruit and stevia beverages slightly less sweet, and more bitter, than aspartame or sucrose. Between 30 and 60 minutes after the preload consumption, fullness was lower, and desire to eat higher, for the three beverages containing non-nutritive sweeteners (NNS) than for the beverage containing sucrose. However there was no significant difference in appetite ratings between the four

beverages after lunch. The sweetener consumed had no effect on thirst, happiness, clear headedness or alertness. Consumption of a beverage containing a NNS was associated with significantly higher energy consumption at lunch than consumption of the beverage containing sucrose, although energy consumption at subsequent meals, after the participant left the research centre, was not significantly different. Mean total daily energy intake was 2330 kcal for aspartame, 2306 kcal for monk fruit, 2241 kcal for stevia and 2312 for sucrose. Energy compensation score was 107% of aspartame, 98% for monk fruit and 73% for stevia. Within the first 60 min after beverage consumption, the sucrose-sweetened beverage was associated with large spikes in blood glucose and blood insulin, whereas the values were relatively stable following consumption of the beverages sweetened with NNS. However the rises in blood glucose and insulin after lunch were greater on days when the participants consumed beverages containing NNS than on the day that they consumed the sucrose-sweetened beverage. Consequently, overall the AUC (area under the curve or total exposure) for blood glucose was the same for all four beverages. The authors concluded that consumption of beverages sweetened with NNS had minimal effects on total daily energy intake, or on postprandial glucose and insulin.

This study was not designed as a tolerance study. However it is noted that no adverse effects were reported as a result of consumption, within 15 min, of 0.63 g monk fruit, equivalent to 0.32 g mogroside V.

3.7 Assessments by other agencies

The Australian Department of Health, Therapeutic Goods Administration (TGA) includes Luo Han Guo material in their list of substances that may be used as listed medicines in Australia. Listed medicines are those classified as being of low risk. Listed medicines may be sold off the shelf in shops such as supermarkets, health food shops and pharmacies, and they include vitamin and mineral supplements, traditional Chinese medicines and herbal medicines. The TGA assesses listed medicines and their ingredients for safety but not for efficacy.

Monk fruit extract is an approved food additive in the Chinese National Standard GB 2760-2014 National Food Safety Standard Food Additive Usage Standard.3. The listing is under the name 'Luohanfruit tincture [*Siraitia grosvenorii* (Swingle) C. Jeffrey]'. Monk fruit extract is classified as a natural flavouring substance permitted in foods, a classification that does not place any restrictions on scope of application, and does not specify maximum allowable concentration levels.

Food additives in Japan are regulated through the Food Sanitation Act 2010. Since 1995, Article 10 of the Act has provided an exclusion from the regulations for 'natural flavouring agents and articles that have generally been served for human consumption and that are used as additives'. Under this exclusion, additives that are widely used and have a long history of human consumption are included in the List of Existing Food Additives, and can be used in food products without restrictions on use or concentration. Monk fruit extract is included on the List of Existing Food Additives, under the name 'rakanka extract'.

Health Canada added monk fruit extract was added to the List of Permitted Sweeteners on December 3, 2013, which allows for its use as a sweetener in table-top sweetener. There are no provisions for its use in other foods in Canada, and such approval would require a formal food additive submission. Health Canada's safety assessment of monk fruit extract was based on a maximum level of use of 0.8% (calculated as mogroside V) in table-top sweeteners, which is the level requested by the petitioner.

The US FDA has approved four GRAS determinations for monk fruit extract as a food additive, in 2010, 2011, 2014 and 2015. All determinations were under the name *'Siraitia*

grosvenorii Swingle (Luo Han Guo) fruit extract', and the US FDA did not have any further questions for the applicants prior to any of the approvals.

4 Discussion

The submitted data are considered adequate to define the hazard of monk fruit extract.

Monk fruit extract is derived from the fruit of *Siraitia grosvenorii*, a perennial vine native to southern China. The sweet components of monk fruit extract are cucurbitane triterpene glycosides known collectively as mogrosides, of which the predominant component of commercial monk fruit extracts is mogroside V, which typically represents 30 to 40% of the extract.

Metabolism studies indicate that mogroside V is largely degraded in the intestinal lumen, with numerous metabolites formed. A number of the metabolites can be measured in plasma, urine, liver and other organs, indicating systemic absorption, but there is also excretion of parent compound and metabolites in the faeces, which suggests that systemic absorption is only partial. Mogroside V is metabolised by a range of reactions, the most common of which are hydroxylation and dehydrogenation.

The available evidence shows that monk fruit extract is not genotoxic, and the LD_{50} in mice could not be established because the toxicity of monk fruit extract is very low. Repeat-dose subchronic studies showed no adverse effects on monk fruit extract at the highest doses tested which were 5 g/kg bw/day in mice, 7.07 g/kg bw/day in male rats, 7.48 g/kg bw/day in female rats, and 3 g/kg bw/d in dogs.

A reproductive and developmental screening study of monk fruit extract, 30% mogroside V w/w, in rats found no adverse clinical or reproductive effects on male or female rats of the P generation, or on F1 pups up to postnatal day 13, of daily doses of monk fruit extract to the P generation up to 4000 mg/kg bw/day. Treatment did not have any demonstrable effects on development or on markers of sexual differentiation or thyroid function in the F1 pups. Soft, sticky faeces in rats dosed with ≥ 2000 mg/kg bw/day were consistent with the low absorption of mogrosides reported in other studies.

No chronic toxicity/carcinogenicity studies are available, but because monk fruit extract is not genotoxic and no lesions that might progress to neoplasia by nongenotoxic mechanisms were observed in subchronic studies, such studies are not considered to be necessary.

Monk fruit is a traditional food and folk medicine in China, and monk fruit extract has a long history of use in Japan. Furthermore monk fruit extract has been available in the USA for a number of years and was recently approved in Canada. No adverse effects on human health or development associated with monk fruit extract consumption in the populations of any of those countries. There is no evidence from human studies that there are any adverse effects of monk fruit consumption, although the studies were not designed as tolerance studies.

5 Conclusions

Based on the reviewed toxicological data, it is concluded that in the absence of any identifiable hazard, an Acceptable Daily Intake (ADI) ‘not specified’ is appropriate for monk fruit extract. A dietary exposure assessment is therefore not required.

6 References

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