ORIGINAL SUBMISSION

JHeimbach LLC

#627

GRN 000627

January 29, 2016

Paulette Gaynor, Ph.D.
Senior Regulatory Project Manager
Division of Biotechnology and GRAS Notice Review (HFS-255)
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740

Dear Dr. Gaynor:

Pursuant to proposed 21 CFR 170.36 (62 FR 18960; April 17, 1997), Guilin GFS Monk Fruit Corporation, through me as its agent, hereby provides notice of a claim that the use of monk fruit juice concentrate in conventional foods, and in infant and toddler foods excluding infant formula, as described in the enclosed notification documents, is exempt from the premarket approval requirement of the Federal Food, Drug, and Cosmetic Act because Guilin GFS Monk Fruit Corporation has determined that the intended use is generally recognized as safe (GRAS) based on scientific procedures.

As required, three signed copies of the notification are provided, including the signed Report of the GRAS Expert Panel. Additionally, I have enclosed a virus-free CD-ROM with the GRAS notice and copies of the signature pages.

If you have any questions regarding this notification, please feel free to contact me at 804-742-5548 or jh@jheimbach.com.

Sincerely,

(b) (6)

James T. Heimbach, Ph.D., F.A.C.N.
President

Encl.

923 Water Street, P.O. Box 66, Port Royal Virginia 22535, USA
tel. (+1) 804-742-5548 fax (+1) 202-478-0986 jh@jheimbach.com

Generally Recognized as Safe (GRAS) Determination of Monk Fruit Juice Concentrate as an Ingredient in Conventional Foods and in Infant and Toddler Foods

Prepared for
Guilin GFS Monk Fruit Corporation
Guilin, China

Prepared by
JHeimbach LLC
Port Royal VA

January 2016

1. GRAS Exemption Claim

Guilin GFS Monk Fruit Corporation ("Monk Fruit Corp"), through its agent JHeimbach LLC, hereby notifies the Food and Drug Administration that the use of monk fruit juice concentrate as an ingredient in conventional foods and in infant and toddler foods, excluding infant formula, as described below, is exempt from the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act because Monk Fruit Corp has determined through scientific procedures that this use is generally recognized as safe (GRAS).

_____ _____

James T. Heimbach, Ph.D., F.A.C.N. Date
President, JHEIMBACH LLC

1.1. Name and Address of Notifier

Guilin GFS Monk Fruit Corporation
5 Liangfeng Road, Yanshan,
Guilin, Guangxi 541006
People's Republic of China

Contact: David Thorrold, General Manager Sales and Marketing
Telephone: +64 7 8496731
Facsimile: +64 7 7 8496730
E-mail: david.thorrold@monkfruitcorp.com

1.2. Name of GRAS Substance

The subject of this GRAS determination is the clarified concentrated juice of monk fruit, *Siraitia grosvenorii* Swingle, also known as luo han guo. A powdered extract of this juice was the subject of GRAS Notice No. GRN 000301, submitted on July 22, 2009. The FDA response letter, stating that the agency had no questions, was dated January 15, 2010. As is discussed further below, the entirety of GRN 301 is incorporated by reference in the present GRAS notice. In the years since GRN 301 was submitted and accepted by FDA, three additional GRAS notices have been submitted and accepted—GRN 359, GRN 522, and GRN 556—and these notices are also cited in this document.

1.3. Intended Use and Consumer Exposure

Monk fruit juice concentrate is intended to be used as a food ingredient, in a manner similar to many other fruit juices, for its flavoring and sweetening properties. It is intended to be used in conventional foods and in infant and toddler foods excluding infant formula.

1.4. Basis for GRAS Determination

Monk Fruit Corp's GRAS determination for the intended use of monk fruit juice is based on scientific procedures as described under 21 CFR §170.30(b).

Determination of the safety and GRAS status of the intended use of monk fruit juice was made through the deliberations of an Expert Panel consisting of individuals qualified by

1. GRAS Exemption Claim

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(b) (6)

1/29/16

James T. Heimbach, Ph.D., F.A.C.N. Date
President, JHEIMBACH LLC

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Guilin GFS Monk Fruit Corporation
5 Liangfeng Road, Yanshan,
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Determination of the safety and GRAS status of the intended use of monk fruit juice was made through the deliberations of an Expert Panel consisting of individuals qualified by

scientific training and experience to evaluate the safety of foods and food ingredients added to foods, including those intended for consumption by infants and toddlers. The Panel included Joseph F. Borzelleca, Ph.D., Berthold V. Koletzko, M.D., and Robert J. Nicolosi, Ph.D. The Panel critically reviewed and evaluated the generally available information and the potential consumption of monk fruit juice concentrate resulting from its intended use, and individually and collectively concluded that no evidence exists in the available information on monk fruit (luo han guo), its juice, juice extracts, or juice concentrate that demonstrates or suggests reasonable grounds to suspect a hazard to consumers under the intended conditions of use of monk fruit juice concentrate.

It is the Expert Panel's opinion that other qualified scientists reviewing the same publicly available data and related information would reach the same conclusion. Therefore, the use of monk fruit juice concentrate under the conditions described is GRAS by scientific procedures.

1.5. Availability of Information

The data and information that serve as the basis for the GRAS determination will be sent to the FDA upon request, or are available for the FDA's review and copying at reasonable times at the office of James T. Heimbach, Ph.D., President, JHeimbach LLC, 923 Water Street, P.O. Box 66, Port Royal, Virginia 22535, telephone 804-742-5548 and e-mail jh@jheimbach.com.

2. Identity of the Substance

Information regarding the identification, characterization, composition, and production of the fruit of *Siraitia grosvenorii* Swingle is contained in GRN 301, which is incorporated by reference. This document includes extensive description of the mogrosides that are the primary difference in the flavor profile of monk fruit juice versus other fruit juices.

The process description in GRN 301 includes the crushing or shredding of the fruit, decoction in 80°C water to extract water-soluble solids, and clarification by passing through a ceramic ultra filtration membrane having a molecular weight cut-off of 100,000 daltons, thus removing protein and pectin from the juice. All of these processing steps are unchanged in the production of monk fruit juice concentrate.

However, the next step in the production of the powdered extract (which is not performed in the production of monk fruit juice concentrate) is filtration of the juice through a column packed with macroporous resin to retain the mogrosides, which are then eluted with an aqueous solution of ethyl alcohol. This step results in the separation of the mogrosides from the sugars and other components that are not adsorbed by the resin and pass through the column.

In the production of monk fruit juice concentrate, instead of passing the juice through an adsorbant column and eluting the retentate, the juice is passed successively through a cationic exchange resin and an anionic exchange resin, thus removing both cationic and anionic substances while leaving the sugars and mogrosides in the juice.

The cationic exchange resin is Dowex Marathon MSC, a sulfonated styrene-divinylbenzene copolymer that complies with 21 CFR §173.25(a)(1), while the anionic resin is Dowex Marathon WBA, a styrene-divinylbenzene and tertiary amine copolymer that complies with 21 CFR §173.25(a)(5). Both resins are food-grade, and, as provided in 21 CFR §173.25, are permitted for use "in the purification of foods, including potable water, to remove undesirable ions."

Monk fruit juice concentrate having a brix level of 65° brix is intended for use in conventional foods as well as infant and toddler foods. The composition of 65° brix monk fruit juice concentrate is shown in Table 1, which also displays the fraction of the juice composition that corresponds to the powdered extract described in GRN 301. It can be seen that the substances found in the powdered extract constitute 8.9% of the 65° brix juice, and most of the remaining material is sugars (56.1%) or water (35.0%). Other substances, including protein (also found in the extract), lipids, fiber, and ash, are each present at less than 0.1%.

Since the components of the powdered extract, including the mogrosides, constitute 8.9% of the juice concentrate, addition of 100 mg of 65° brix juice concentrate to foods provides the same concentration of these substances as addition of 8.9 mg of the extract—i.e., a ratio of 11.24:1.

Table 1. Composition of 65° brix Monk Fruit Juice Concentrate.

Component	% (w/w) of Monk Fruit Juice Concentrate	% (w/w) of Monk Fruit Juice Extract
Substances in extract	8.9	100.0
Mogroside V	3.5	39.0
Other mogrosides	1.5	16.2
Protein	0.1	1.1
Other[1]	3.7	43.7
Total sugars	56.1	
Sucrose	31.3	
Glucose	13.9	
Fructose	10.9	
Lactose	<0.1	
Maltose	<0.1	
Lipid	<0.1	
Fiber	<0.1	
Ash	<0.1	
Moisture	35.0	

1. Other components are primarily melanoidins and flavonoids.

All production of monk fruit juice concentrate is performed under current Good Manufacturing Practice (cGMP). Monk Fruit Corp has established specifications for food-grade monk fruit juice concentrate and analyzed 3 non-consecutive lots of product to confirm that the production process is in control and consistently results in the manufacture of food-grade product. The specifications and results of analyses are shown in Table 2. As can be seen, all tested samples conformed with the specifications for food-grade material.

Table 2. Food Grade Specifications for Monk Fruit Juice Concentrate.

Parameter	Unit	Specifi-cation	Tested Lots		
			45GFL-1503015	45GFL-1504017	45GFL-1504018
Mogroside V	% (w/w)	3.5±0.2	3.39	3.36	3.44
Brix	º	65.0 – 70.0	66.7	65.1	65.1
Turbidity (at 10º brix)	NTU[1]	≤10	3.2	1.5	4.4
Color absorbancy (at 10º brix, 440 nm)		≤0.15	0.053	0.044	0.059
Heavy metals					
Arsenic	mg/kg	≤0.4	≤0.4	≤0.4	≤0.4
Lead	mg/kg	≤0.5	≤0.5	≤0.5	≤0.5
Microbiology					
Total plate count	cfu[2]/g	<1000	1	1	1
Total yeast	cfu/g	<20	<1	<1	<1
Total mold	cfu/g	<20	<1	<1	<1
Coliforms	cfu/g	<1	<1	<1	<1
Pathogenic bacteria	cfu/5 g	Negative	Complies	Complies	Complies

1. NTU = nephelometric turbidity unit
2. cfu = colony-forming unit

3. Intended Use and Exposure

Monk fruit juice concentrate is intended for use as a food ingredient to be added to foods in order to provide the flavor and sweetness of monk fruit, a use similar to that of other fruit juices and concentrates. Also like other fruit juices and concentrates, the level of addition of monk fruit juice concentrate is limited only by cGMP. Practically, this results in a maximum addition level of about 1%, more frequently 0.25 – 0.50%. As was described in the preceding section, monk fruit juice concentrate differs from the powdered extract that was the subject of GRAS notice GRN 301 in retaining the sugars and water that are removed in producing the powdered extract. Mogrosides and other components that are also present in the powdered extract constitute 8.9% of the juice when concentrated to 65° brix. Consequently, addition of the juice to foods at the level needed to provide the same amount of mogrosides as is provided by the powdered extract requires addition of 11.24 mg of 65° brix juice to replace each 1.00 mg of powdered extract (1/0.089).

Monk fruit juice concentrate having a brix level of 65º brix concentrate is intended for use in conventional foods as well as infant and toddler foods (excluding infant formula).

In GRN 301, the mean daily intake of the powdered extract in the general population was estimated at 2.6 mg/kg bw, while the estimated 90th percentile intake was 6.8 mg/kg bw/day; the estimated 90th percentile intake among children was 9.9 mg/kg bw/day. Intakes of 65° brix monk fruit juice may be estimated as 11.24 times these levels, or a daily mean of 29.3 mg/kg bw and 90th percentile of 76.4 mg/kg bw. This is a generous estimate, because—although the addition level of monk fruit juice concentrate is limited only by cGMP—its most likely uses are to substitute for other fruit juices in foods and beverages sweetened and flavored by the addition of fruit juice or fruit juice concentrate, a limited market.

The exposure of the powdered extract components is unchanged from that calculated in GRN 301, an exposure that was determined to be both safe and GRAS. The additional exposure of the juice is due to sugars (sucrose, glucose, and fructose) and water, GRAS components with no safety issues.

The intended use of monk fruit juice concentrate in infant and toddler foods (excluding infant formula) is primarily in fruit-containing baby foods and infant cereals. Based on data provided by a manufacturer of these foods, consumption of these foods is as shown in Table 3. The median bodyweights for boys and girls are derived from growth charts from the Centers for Disease Control and Prevention, and the estimated daily consumption figures are based on 95th percentile consumption divided by bodyweight.

Table 3. Consumption of Baby Food with Fruit and Infant Cereal

Age (Months)	Median Weight (kg)	Baby Food with Fruit		Infant Cereal		95th %ile Baby Food Consumption (g/kg bw/day)	95th %ile Infant Cereal Consumption (g/kg bw/day)
		Mean (g/day)	95th %ile (g/day)	Mean (g/day)	95th %ile (g/day)		
4-5.9	7.2	80	*	17	*	15.7	15.7
6-8.9	8.2	109	113	19	113	13.8	13.8
9-11.9	8.9	128	170	27	113	19.1	12.7
12-14.9	9.5	102	170	22	123	17.9	12.9
15-17.9	10.7	†	170	†	205	15.9	19.2

* Insufficient data; 95th percentile from age 6-8.9-month group used for consumption estimate.
† Data not available.

As noted above, the intended use of monk fruit juice concentrate in infant and toddler foods (excluding infant formula) is at an addition level of 0.25 to 0.5%. At the latter addition level of 0.5%, the 95th percentile intake of monk fruit juice concentrate is as shown in Table 4.

Table 4. Estimated Intake of Monk Fruit Juice Concentrate Added at 0.5% to Baby Food with Fruit and Infant Cereal.

Age (Months)	95th %ile from Baby Food with Fruit (mg/kg bw/day)	95th %ile from Infant Cereal (mg/kg bw/day)
4-5.9	78.5	78.5
6-8.9	68.9	68.9
9-11.9	95.5	63.5
12-14.9	89.5	64.7
15-17.9	79.4	95.8

FDA (2006) suggests that "consumption at the 90th percentile for most commonly-consumed foods is approximately 2 times the mean consumption for that food, and intake at the 95th percentile is approximately 4 times the mean." This would imply that the 95th percentile estimates in Table 4 are about twice as high as 90th percentile estimates would be, indicating that the highest 90th percentile estimate of intake of monk fruit juice concentrate from baby food would be 47.8 mg/kg bw/day among 9-11.9-month olds and that from infant cereal would be 47.9 mg/kg bw/day among 15-17.9-month olds. About 5% of this intake is mogrosides; thus, the highest estimated 90th percentile intake of mogrosides from either baby food or infant cereal is 2.4 mg/kg bw/day. This is substantially lower than the 9.9 mg/kg bw/day estimated 90th percentile intake of monk fruit juice extract reported in GRN 301.

4. Safety

4.1. Monk Fruit Extract Described in GRN 301

This section of GRN 301 is incorporated by reference. It clearly demonstrates the safety and GRAS status of the substances present in the powdered extract that was the subject of that GRAS notice. The remaining components of the monk fruit juice concentrate are primarily sucrose, glucose, fructose, and water, all GRAS substances with no significant safety issues.

4.2. Monk Fruit Extract Described in GRN 359

In the years since GRN 301 was submitted and accepted by FDA, three additional GRAS notices have been submitted—GRN 359, GRN 522, and GRN 556—and all have been accepted. The first of these, GRN 359, received by FDA on November 4, 2010, concerned an extract produced by Guilin Layn Natural Ingredients Corp.; FDA's response indicating "no questions at this time" was dated April 11, 2011 (FDA 2011). This GRAS submission noted that the subject of the notice is "similar in composition to BioVittoria's PureLo® Luo Han Fruit concentrate, a material previously affirmed GRAS (GRN 000301) with no questions from the US FDA CFSAN/Office of Food Additive Safety," and is intended for the same use, but the GRAS determination featured a confirmation of safety and GRAS status from an Expert Panel comprising three scientists different from those who reviewed PureLo®.

The GRN 359 submission included a report of an unpublished subchronic (90-day) feeding study of the oral toxicity of the monk fruit extract that was the subject of the GRAS notice. The description of the study is incorporated by reference but quoted here for convenience:

> "A 90-day oral toxicity study of Go-LuoTM5 5% powder extract was conducted at Huntingdon Life Sciences in Crl:CD@ (SD) IGS BR rats. The study was carried out in accordance with: Part 58 of 21 CFR (FDA Good Laboratory Practice Regulations) and current Good Laboratory Practice (GLP).
>
> "Five- to six-week-old rats (Charles River Laboratories, Raleigh, North Carolina) were acclimatized to housing facilities for approximately two weeks prior to being placed into treatment groups. Animal room controls were set to maintain room temperature at approximately 18 to 26ºC, relative humidity of 30 to 70%, and a light-dark cycle of 12 hours each. Animals received a commercially available laboratory rodent diet (PMI Nutrition International, St. Louis, Missouri) and drinking water *ad libitum.* Animals were assigned to groups by a computerized stratified randomization program in order to have comparable body weight means for each group. Crl:CDB (SD) rats (20 animals/sex/group) for the main study groups, plus an additional 10 animals/sex/group for dietary control and high-dose recovery groups, were fed with 0 (control), 12,500, 25,000, or 50,000 ppm Go-Luo™ 55% powder extract in the diet for 90 days. Animals were housed individually for food consumption and body weight determination. Fresh diets were prepared and provided on a weekly basis. Animals were examined twice daily for mortality and morbidity. Food consumption and body weights were measured weekly throughout the study. Ophthalmological examinations were performed during Pre-test Week 2, Week 13, and Recovery Week 5.
>
> "At the end of the treatment period, animals from the main study (20 animal/sex/group) were euthanized and necropsied. The remaining animals (10/sex/group) from the control and high-dose groups were held for a 28-day treatment-free recovery period before being euthanized and necropsied. Animals were fasted overnight prior to blood collection. Blood samples were analyzed for the following hematological parameters: hemoglobin

concentration (HGB), hematocrit (HCT), red blood cell (RBC) count, platelet (PLT) count, mean platelet volume (MPV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), white blood cell (WBC) count, absolute reticulocyte (RETIC) count. Prothrombin time (PT), activated partial thromboplastin time (APTT), and fibrinogen (FIB) were measured using a blood coagulation analysis apparatus.

"Blood for biochemical examinations was collected into tubes with no anticoagulant, allowed to clot, and centrifuged to obtain serum. Serum samples were analyzed for the following: alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, urea nitrogen, creatinine, glucose, total cholesterol, triglycerides, total protein, albumin, globulin (calculated as total protein - albumin = globulin), albumin/globulin ratio (calculated), bilirubin (total), sodium, potassium, chloride, calcium, and phosphorus.

"Urine was collected (20/sex/group at week 13; 10/sex/group in groups 1 and 4 during week 18) and evaluated for volume (16-hour), specific gravity, appearance, pH, nitrites, protein, glucose, ketones, urobilinogen, bilirubin, and urine chemistry (creatinine, phosphorus, and calcium).

"At the time of necropsy, the following organs were removed and weighed for all animals: adrenal glands, heart, brain (medulla, pons, cerebrum, and cerebellum), kidneys, liver, ovaries, spleen, testis, epididymides, pituitary, prostate/seminal vesicles, thyroid with parathyroid glands, uterus (with cervix) and thymus. Paired organs were weighed together.

"The following organs/tissues were collected from all animals: heart, aorta, lung, airway, liver, pancreas, tongue, salivary gland (sublingual gland, submandibular gland), gastrointestinal tract (esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum), thymus, spleen, lymph node (mesenteric lymph node), kidney, bladder, male genital organs (testes, epididymis, seminal vesicle, prostate), female genital organs (ovarium, uterus, vagina), mammary gland, pituitary gland, adrenal gland, thyroid gland, parathyroid, brain (cerebrum, cerebellum, medulla, pons), skin, eyes, accessory gland (Harderian gland), bone and bone marrow (sternum, femur). All tissue samples were fixed in 10% neutral-buffered formalin. Eyes and testes were placed in Modified Davidson's solution and then retained in 10% formalin.

"No treatment-related mortality was observed. Four male rats (one from each of the four dose groups) were humanely euthanized or found dead. Based on their sporadic nature and the absence of any similar pathology in the terminal animals, none of these deaths were considered to be related to Go-LuoTM 55% powder extract administration. Ophthalmological examination revealed no abnormalities at the end of the dosing and recovery periods. There were no statistically significant differences in body weight or food consumption during the dosing and recovery periods.

"Hematological results showed no remarkable effects. There was a slight increase in MPV at ≥25,000 ppm in animals of both sexes; slightly increased MCH and slightly decreased RDW in 50,000 ppm males; and slightly increased circulating lymphocyte counts in ≥25,000 ppm females. However, all these parameters were within historical control variation. Serum chemistry results were also unremarkable. Both sexes at ≥12,500 ppm had a slight decrease in triglyceride values and females in the same dose-group had a slight decrease of total bilirubin. These changes were also within historical control variation.

> "There were no histopathological observations in any tissue samples or organs. A slight increase in the absolute and relative liver weights was noted in ≥12,500 ppm females which appeared to be an adaptive response and was non-adverse. The results of the oral toxicity study showed no adverse effects in rats receiving 12,500, 25,000, and 50,000 ppm of Go-Luo™ in the diet for 90 days. Although some variations in hematology, clinical chemistry, and in female liver weights were observed, none of these findings were considered treatment-related. Therefore, the no-observed-adverse-effect level (NOAEL) for Go-Luo™ 55% powder extract was considered to be a dietary concentration of 50,000 ppm. This was equivalent to a time-weighted average dose over the course of the dosing period of approximately 3.12 g/kg bw/day and 3.75 g/kg bw/day in male and female rats, respectively" (GRN 359).

This unpublished study further corroborates the safety of monk fruit juice, monk fruit juice concentrate, and its even more concentrated extracts.

In addition to the subchronic oral toxicity study, the GRN 359 notice also included a report of an unpublished bacterial reverse mutation test of the same substance. Again, while this report is incorporated by reference, it is repeated here for convenience:

> "A bacterial reverse mutation test or Ames Test was conducted at Huntingdon Life Sciences to assess Go-LuoTM 55% powder extract for its ability to cause point (gene) mutation in *Salmonella typhimurium* strains TAI 535, TAI 537, TA98, TAI 00 and *Escherichia coli* strain WP2*uvr*A. This study was conducted in compliance with the OECD Guideline for the Testing of Chemicals, Number 471 (Genetic Toxicology: Bacterial Reverse Mutation Test); current Good Laboratory Practice (cGLP); EC Commission Directive 2000/32/EC Annex 4D-B. 13/14 (Mutagenicity- Reverse mutation test in bacteria); EPA Health Effects Test Guidelines (OPPTS 870.51 00 Bacterial reverse mutation test) and FDA Redbook 2000 (Bacterial-Reverse Mutation Test).
>
> "Five concentrations separated by approximately half-$\log_{10}$ intervals were tested, with a maximum of 5000 µg (of mogroside V) per plate (i.e., 9090 µg Go-Luo™ 55% powder extract). No cytotoxic activity was observed at the concentrations assayed. In addition to test article, strains were assayed in the presence of an aqueous negative control and in the presence of sodium azide, 9-aminoacridine, 2-nitrofluorene, and 4-nitroquinoline-1-oxide positive controls. These tests were undertaken in the absence of S9 mix. Moreover, strains were assayed in the presence of S9 mix plus test article at the identical concentrations, plus aqueous negative control, and 2-aminoanthracene and benzo[a]pyrene positive controls. There were no substantial increases in revertant colony numbers over aqueous control counts at any concentration up to 5000 µg/plate in the tested bacterial strains, either in the presence or absence of S9 mix. Under the test conditions employed, Go-LuoTM 55% powder extract did not exhibit any cytotoxic or mutagenic potential" (GRN 359).

4.3. Monk Fruit Extract Described in GRN 522

GRAS Notice (GRN) 522 was submitted to FDA on May 21, 2014 and filed on June 9, 2014. FDA had no questions regarding the safety or GRAS status of the monk fruit extract described in this notice, as noted in the Agency's response letter dated December 8, 2014. GRN 522 reported that on December 2, 2013, Health Canada had added monk fruit extract to the *List of Permitted Sweeteners* for use as a table-top sweetener at a maximum use level of 0.8% calculated as mogroside V. An Expert Panel, comprising three scientists not participating in either GRN 301 or GRN 339, determined that the intended use of the monk fruit extract

described in GRN 522—which duplicates the uses listed in the previous GRAS notices—is safe and GRAS by scientific procedures.

4.4. Monk Fruit Extract Described in GRN 556

The fourth GRAS notice for monk fruit extract, GRN 556, was filed by FDA on December 29, 2014; the Agency's response letter, stating that FDA had no questions at that time, was sent on June 17, 2015. The intended use of monk fruit extract in GRN 556 was again the same as originally specified in GRN 301, and the safety and GRAS status of this use was assessed by an Expert Panel that included two scientists not involved with earlier review of monk fruit juice or its extracts. The material presented in GRN 556 included monk fruit extracts that contained a crude decoction of monk fruit containing 3% mogroside V, providing further support for the safety of the non-mogroside components of monk fruit.

4.5. Summary of Monk Fruit Extract GRAS Notices

In the four GRAS notices for monk fruit extracts submitted to date, the concentrations of mogrosides (expressed as mogroside V) ranged from 12.5% to 90%. Since the mogroside V content of 65º brix monk fruit juice is about 3.5%, and the total mogroside content is about 5.0%, the least concentrated extract is only concentrated by a factor of about 3, while the most concentration extract is concentrated by a factor of about 20.

The intended use of all monk fruit extracts discussed in the GRAS notices is as general-use sweeteners and flavor modifiers, permitted both for table-top sweetener use and as ingredients in all foods in which such use is not prohibited by standards of identity, other than infant formulas or meat and poultry products. Permitted uses thus include infant and toddler foods other than infant formula.

The estimated daily intakes (EDI) of monk fruit extracts were reported as ranging from 2.41 mg/kg bw/day for the 90% extract to 12.4 mg/kg bw/day for the 12.5% extract. In GRN 556, the EDI of mogroside V among healthy children was reported as 2.17 mg/kg bw/day. In GRN 301, in which the monk fruit extract was less highly concentrated, the intake of the extract among healthy children was very conservatively estimated at the mean and 90th percentile as 4.2 and 9.9 mg/kg bw/day, respectively.

4.6. Additional Research on Monk Fruit Juice and Mogrosides

Some recent research has served to further elucidate the intestinal handling of mogrosides, particularly mogroside V, the principal mogroside in monk fruit juice and its extracts. Murata et al. (2010) studied the digestion and absorption of monk fruit mogrosides in Wistar rats. A laboratory-made spray-dried extract of monk fruit juice was determined by high performance liquid chromatography (HPLC) to be 72.0% mogroside V. Two groups of 10-week-old Wistar rats (number, sex, and bodyweight not reported) were fasted for 16 hours and gavaged with 117 mg of the extract. Portal blood, whole blood, and small-intestine contents were collected from one group of rats after 120 minutes, while urine and feces were collected from the second group after 24 hours; samples were analyzed for mogroside V and its metabolites using liquid chromatography-mass spectrometry.

Little of the mogroside was absorbed. While traces of mogroside IE and of mogrol were found in portal blood, no mogroside V, nor any of the mogrosides with 2, 3, or 4 glucose

residues were detected. No mogrol or mogrosides were detected in whole blood or in urine. Over 70% of the small-intestine triterpenoid content was mogroside V; 22% was mogroside IV and 5% was mogroside III. The mogroside III and IV most likely resulted from deglycosylation of mogroside V although they may also have been present in the 28% of the administered test article that was not mogroside V. Much more extensive deglycosylation was evident in the mogrol and mogrosides detected in feces, likely indicating fermentation by colonic bacteria. Over 60% of the administered dose of mogroside V was found in feces, 49% in the form of mogroside II and 48% as mogrol. Murata et al. (2010) concluded that "the absorbed amount of [mogroside V] and its metabolites was extremely low" and that "most of the orally ingested mogroside V is excreted without absorption."

Murata et al. (2010) additionally concluded that the findings "suggest that mogroside IIA can be produced only by intestinal microflora." This conclusion supports the earlier findings of Yang et al. (2007), who incubated mogroside III with crude enzymes of human colonic bacteria in an *in vitro* study and found that it was converted to mogroside IIA and mogrol by successive deglycosylation at carbon 3 and carbon 24.

Using more sophisticated analytical techniques than previous work, Xu et al. (2015) studied the metabolism of mogroside V in 2 *in vitro* sytems—rat hepatic supernatant (S9) incubation and human intestinal bacteria incubation—and *in vivo* in the rat. The method employed, HPLC in tandem with electrospray-ionization ion-trap time-of-flight mass-spectrometry (HPLC-ESI-IT-TOF-MS^n), identified 77 metabolites that had not previously been identified, including 52 oxidation products formed by mono- to tetra-hydroxylation/dehydrogenation of mogroside V.

For the rat hepatic supernatant study, hepatic S9 samples were prepared from 6 rats and placed in 2 incubators, to one of which was added mogroside V while the other served as an S9 control. The only metabolites of mogroside V detected in this system, found at very low level, were 4 isomers of mogroside VI, indicating that the rat hepatic enzymes were capable of glucosylation of the mogroside, presumably utilizing the small amount of free glucose available in the test article.

Feces from a healthy man were subjected to 48 hours of anaerobic incubation at 37ºC to obtain activated intestinal bacterial biota. As with the S9 study, the human intestinal bacteria were placed in 2 incubators, to only one of which was added mogroside V. The metabolites detected in the human colonic bacterial system were all products of glucosylation or deglucosylation—one mogroside VI isomer, 2 mogroside V isomers, 3 mogroside IV isomers, 2 mogroside II isomers, and 2 mogroside I isomers. As was the case with the S9 system, no oxidative metabolites were detected, but human colonic microbiota appear to be capable of isomerization and hydrolysis of the mogrosides as well as glucosylation and deglucosylation.

For the *in vivo* study, Xu et al. (2015) placed 8 male Sprague-Dawley rats weighing 230±20 g (age not reported) in metabolic cages with feed and water available *ad libitum*. Four rats served as controls, while the other 4 were gavaged with 50 mg mogroside V/kg bw/day for 4 days. On the final day, 1 hour after dosing, fecal, blood, and organ samples (heart, liver, spleen, lungs, kidneys, stomach, and small intestine) were obtained from rats of both groups and analyzed for mogroside V and its metabolites.

About 60% of the mogrol and mogroside detected in the organs of the rats was found in the small intestine and 28% in the stomach. Approximately 16% was found in the liver, and only

1% or less in other organs. Relatively small amounts of mogroside metabolites were detected in plasma or urine, much of them (30% in urine and 51% in plasma) unchanged as mogroside V, while none of the material found in feces was unchanged mogroside V and 15% was fully deglucosylated mogrol. The concentration of mogroside metabolites in feces was more than 95 times greater than in plasma or urine. The authors concluded that "The metabolic reactions of mogroside V include deglycosylation, hydroxylation, dehydrogenation, isomerization, glucosylation, and methylation." Their data further show that nearly all ingested mogroside V is rapidly excreted, almost entirely in feces, and primarily in deglucosylated forms including mogroside III, mogroside II, and mogrol.

5. Safety Assessment and GRAS Determination

5.1. Introduction

This section presents an assessment that demonstrates that the intended use of monk fruit juice concentrate is safe, and is GRAS by scientific procedures. This safety assessment and GRAS determination entail two steps. In the first step, the safety of monk fruit juice concentrate under its intended conditions of use is demonstrated. Safety is established by demonstrating a reasonable certainty that the exposure of consumers, including infants and toddlers, to monk fruit juice concentrate under its intended conditions of use is not harmful. In the second step, the intended use of monk fruit juice concentrate is determined to be GRAS by demonstrating that the safety of this concentrate under its intended conditions of use is generally recognized among qualified scientific experts and is based on publicly available and accepted information.

5.2. Safety Evaluation

Numerous studies in both rodents and non-rodents, as well as in human adults, individually and collectively demonstrated the safety of monk fruit itself as well as juice concentrate and extracts. This body of evidence was presented and evaluated in GRN 301, which—as has already been stated—is incorporated by reference. Corroborative data were offered in GRN 359, which is also incorporated by reference. More recent investigations of the absorption, metabolism, and excretion of mogrosides, particularly mogroside V, confirm that absorption of mogrosides is low, and most of the little that is absorbed is rapidly excreted.

5.3. General Recognition of the Safety of Monk Fruit Juice Concentrate

The intended use of monk fruit juice concentrate has been determined to be safe through scientific procedures as set forth under 21 CFR §170.30(b). This safety was shown by establishing the identity and characteristics of the substance; demonstrating the similarity of monk fruit juice concentrate to other GRAS powdered extracts; finding that consumption of monk fruit juice concentrate does not lead to adverse effects; and concluding that the expected exposure to monk fruit juice concentrate is without significant risk of harm. Finally, because this safety assessment satisfies the common knowledge requirement of a GRAS determination, this intended use can be considered GRAS.

Determination of the safety and GRAS status of the intended use of monk fruit juice has been made through the deliberations of an Expert Panel consisting of Joseph F. Borzelleca, Ph.D., Berthold V. Koletzko, M.D., and Robert J. Nicolosi, Ph.D., who reviewed a monograph prepared by JHeimbach LLC as well as other information available to them. These individuals are qualified by scientific training and experience to evaluate the safety of foods and food ingredients, including those intended for consumption by infants and toddlers. They critically reviewed and evaluated the publicly available data, related information, and potential exposure to monk fruit juice concentrate anticipated to result from its intended use, and determined that no evidence exists in the available information on monk fruit, monk fruit juice, its powdered extract, or monk fruit juice concentrate that demonstrates or suggests reasonable grounds to suspect a hazard to consumers under the intended conditions of use.

6. References

The reference list from GRN 301 is incorporated by reference. Additional references are listed below.

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Report of the Expert Panel on the Generally Recognized as Safe (GRAS) Determination of Monk Fruit Juice Concentrate as an Ingredient in Conventional Foods and in Infant and Toddler Foods

Prepared for
Guilin GFS Monk Fruit Corporation
Guilin China

Prepared by
JHeimbach LLC
Port Royal VA

January 2016

Report of the Expert Panel on the Generally Recognized as Safe (GRAS) Determination of Monk Fruit Juice Concentrate as an Ingredient in Conventional Foods and in Infant and Toddler Foods

We, the members of the Expert Panel, have individually and collectively critically evaluated the publicly available information on monk fruit juice concentrate summarized in a monograph prepared by JHEIMBACH LLC, as well as other material deemed appropriate or necessary. Our evaluation included review of the identity and physical-chemical properties of monk fruit juice concentrate, including production methods, the potential exposure resulting from its intended use, published research bearing on its safety, and GRAS notices for monk fruit juice extracts submitted to and reviewed by the Food and Drug Administration (FDA). Our summary and conclusion resulting from this critical evaluation are presented below.

Summary

- The subject of this GRAS determination is the clarified concentrated juice of monk fruit, *Siraitia grosvenorii* Swingle, also known as luo han guo. At 65º brix, this juice concentrate contains about 3.5% mogroside V, 1.5% other mogrosides, and 56.1% nutritive sugars. It is about 35% water.
- Extracts of monk fruit juice have been the subject of four GRAS notifications to FDA: GRN 301 (submitted July 22, 2009), GRN 359 (November 3, 2010), GRN 522 (May 21, 2014), and GRN 556 (November 24, 2014). FDA responded to all of these notices that the Agency had no questions regarding them. The mogroside concentrations in the extracts described in these GRAS notices ranged from 12.5 to 90%.
- Production of monk fruit juice concentrate includes the following steps: crushing the fruit, extracting water-soluble solids by decoction in 80ºC water, clarifying the juice by ultra-filtration, and removing anionic and cationic substances with approved food-grade ionic exchange resins.
- Monk fruit juice concentrate is intended to be used as a food ingredient, in a manner similar to many other fruit juices, for its flavoring and sweetening properties. It is intended to be used in conventional foods and in infant and toddler foods excluding infant formula.
- The level of addition of monk fruit juice concentrate is limited only by current Good Manufacturing Practice (cGMP), but in practice addition is limited to about 1%, and more frequently 0.25 to 0.5%, due to adverse organoleptic characteristics at higher levels.
- The estimated mean and 90th-percentile daily intakes of monk fruit juice concentrate from its intended use in conventional foods (which includes consumption by all Americans aged 2 years and older) are 29.3 and 76.4 mg/kg bw/day, respectively; over 90% of these intakes are water and nutritive sugars and about 5% is mogrosides. The estimated mean and 90th-percentile daily intakes of mogrosides from the intended use of monk fruit juice concentrate in conventional foods are thus 1.5 and 3.8 mg/kg bw/day, respectively, much less than the estimated intakes of mogrosides from monk fruit juice extracts in GRAS notices provided to

FDA (6.8 mg/kg bw/day for adults and 9.9 mg/kg bw/day for children), which were determined to be safe and GRAS.

- The highest estimated 90th-percentile intake of mogrosides from the intended use of monk fruit juice concentrate in baby foods with fruit is 2.4 mg/kg bw/day among infants 9-12 months old, while that from the intended use of monk fruit juice concentrate in infant cereal is 2.4 mg/kg bw/day among toddlers 15-18 months old.
- The safety of the intended use of monk fruit juice concentrate is supported by published oral toxicity studies of the extract described in GRN 301 (incorporated in this GRAS determination by reference) and by an unpublished subchronic oral toxicity study and a bacterial reverse mutation test described in GRN 369 (incorporated by reference).
- The safety of the intended use of monk fruit juice concentrate is further supported by published human studies described in GRN 301 (incorporated by reference) and the other GRAS notices for monk fruit juice extracts.
- In a study in which Wistar rats were gavaged with an extract of monk fruit juice containing 72% mogroside V, it was reported that traces of mogroside IE and of mogrol were found in portal blood, but no mogroside V, nor any of the mogrosides with 2, 3, or 4 glucose residues were reported. No mogrol or mogrosides were reported in whole blood or in urine. Over 60% of the administered dose of mogroside V was found in feces, 49% in the form of mogroside II and 48% as mogrol.
- In another study in which both *in vitro* and *in vivo* methods were used to explore the metabolism of mogroside V, the findings showed that metabolic reactions of mogroside V include deglycosylation, hydroxylation, dehydrogenation, isomerization, glucosylation, and methylation, but that nearly all ingested mogroside V is rapidly excreted, almost entirely in feces, primarily in deglucosylated forms.

Conclusion of the Expert Panel

We, the undersigned independent Expert Panel members, individually and collectively critically evaluated the data and information summarized above, and unanimously conclude that the addition of monk fruit juice concentrate produced in accordance with current Good Manufacturing Practice and meeting appropriate food-grade specifications to conventional foods and to infant and toddler foods other than infant formula as described is safe.

We further conclude that the addition of monk fruit juice concentrate produced in accordance with current Good Manufacturing Practice and meeting appropriate food-grade specifications to conventional foods and to infant and toddler foods other than infant formula as described is generally recognized as safe (GRAS) based on scientific procedures.

It is our unanimous opinion that other qualified experts would concur with these conclusions.

_____ _____

Joseph F. Borzelleca, Ph.D. Date
Emeritus Professor of Toxicology and Pharmacology
Virginia Commonwealth University School of Medicine
Richmond, Virginia, USA

_____ _____

Berthold V. Koletzko, M.D. Date
Professor of Pediatrics
University of Munich
Munich, Germany

_____ _____

Robert J. Nicolosi, Ph.D. Date
Emeritus Professor of Clinical Laboratory and Nutritional Sciences
University of Massachusetts—Lowell
Lowell, Massachusetts, USA

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It is our unanimous opinion that other qualified experts would concur with these conclusions.

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25 January 2016

Joseph F. Borzelleca, Ph.D. — Date
Emeritus Professor of Toxicology and Pharmacology
Virginia Commonwealth University School of Medicine
Richmond, Virginia, USA

Berthold V. Koletzko, M.D. — Date
Professor of Pediatrics
University of Munich
Munich, Germany

Robert J. Nicolosi, Ph.D. — Date
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Joseph F. Borzelleca, Ph.D. — Date
Emeritus Professor of Toxicology and Pharmacology
Virginia Commonwealth University School of Medicine
Richmond, Virginia, USA

(b) (6)

26. Jan. 2016
Berthold V. Koletzko, M.D. — Date
Professor of Pediatrics
University of Munich
Munich, Germany

Robert J. Nicolosi, Ph.D. — Date
Emeritus Professor of Clinical Laboratory and Nutritional Sciences
University of Massachusetts—Lowell
Lowell, Massachusetts, USA

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Joseph F. Borzelleca, Ph.D. — Date
Emeritus Professor of Toxicology and Pharmacology
Virginia Commonwealth University School of Medicine
Richmond, Virginia, USA

Berthold V. Koletzko, M.D. — Date
Professor of Pediatrics
University of Munich
Munich, Germany

(b) (6)

Robert J. Nicolosi, Ph.D. — Date 1/26/2016
Emeritus Professor of Clinical Laboratory and Nutritional Sciences
University of Massachusetts—Lowell
Lowell, Massachusetts, USA

SUBMISSION END