This document and the related attachments are in response to the questions provided by the FDA regarding GRAS Notice GRN 000901, dated May 4, 2020. They are presented in the same order and under the same section names as stated in the FDA letter. The FDA questions are in blue font with the responses following in black.

Chemistry review

1. On pages 11–12, the notifier states that the substances used in the manufacture of glucosyl hesperidin are suitable for food production and are used in accordance with Food Sanitation Act of Japan. Please provide a statement to confirm that the substances and materials, such as processing aids and ion exchange resins, meet applicable U.S. regulations or are GRAS for the intended use.

Response: The following information includes the regulatory basis for the raw materials and processing aids used in the production of GH. All, except the ion exchange resins, meet applicable US regulations, are GRAS for the intended use, or have been reviewed by a Select Committee on GRAS Substances (SCOGS). The ion exchange resins are certified by the supplier to meet the monograph in Japan's Specifications and Standards for Food Additives of the Food Sanitation Law (see attached certificate from supplier titled "Ion Exchange resins"). However, the supplier could not certify that the resins meet applicable U.S. regulations.

Hesperidin	Substances Added to Food (formerly EAFUS) – as a Flavor enhancer, Nutrient supplement. SCOGS II-3, 1982, Evaluation of Health Aspects of Hesperidin, Naringin, and Citrus Bioflavonoid Extracts as Food Ingredients. GRN 000796, Orange extract (85% hesperidin). Comment – While GRN 000796 provides hesperidin is used as a bioflavonoid in various foods and not specifically for an ingredient for further manufacture it does suggest that it is thought to be safe for use.
Dextrin	21CFR §184.1277
Ascorbic acid	21CFR §182.3013
Sodium hydroxide	21CFR §184.1763
Magnesium chloride	21CFR §184.1426
Hydrochloric acid	21CFR §182.1057
Sodium pyrosulfite	21CFR §182.3739
Sulfuric acid	21CFR §184.1095
Sodium chloride	21CFR §182.1(a)
Ethanol	21CFR §184.1293
Activated carbon	SCOGS II-6, 1981, Evaluation of the Health Aspects of Activated Carbon (Charcoal) as a Food Processing Aid.
Diatomaceous earth	SCOGS 61, 1979, Silicates, Diatomaceous earth (filter aid).
Perlite	SCOGS 61, 1979, Silicates, Perlite (filter aid).
Powdered cellulose	GRAS by prior use 1958.
Ion exchange resins	Japan's Specifications and Standards for Food Additives of the Food Sanitation Law. Amberlyte™ IRA96SB and Amberlyst™ 16WET
Adsorption separation resin	21CFR §177.2420
Glucoamylase	21CFR §173.130; §172.892.
Cyclodextrin glucanotransferase	GRN 000405, and GRN 000046, 00074 & 000155.

2. On page 31, the notifier provides a description of the intended use and includes "fruit and fruit flavored drinks," whereas, page 105 (Table 1) lists "fruit flavored drinks or fruit juice and powders." We note that the list of foods included as part of the estimated dietary exposure determination did not include fruit, fruit juices, nectars or blends (for example, food codes starting 612* (citrus juices) and 641* (non-citrus juices and nectars). Please confirm whether the intended use includes all fruit juices and nectars or if the intended use is limited to fruit-flavored drinks.

Response: The listing on page 31 of intended uses for "fruit and fruit flavored drinks", as compared to Table 1 on page 105 for "fruit flavored drinks or fruit juices and powders is partially incorrect. This category should have been stated on page 31 as "fruit juice drinks, and fruit flavored drinks", and on page 105 "fruit flavored drinks, and fruit juice drinks and powders". As can be seen from the food codes provided there are no "citrus juices" (*612) or "non-citrus juices" (*641) requested as intended uses.

3. We noted some apparent discrepancies in the Appendix titled "Food Codes Under the Intended Uses" (pages 115–120). There is a mismatch between food codes and their descriptions beginning at food code 91361020, which is described as "milk chocolate candy, plain," however, this code corresponds to "fruit sauce." Although not exhaustive, the food codes and descriptions listed below are missing their corresponding code or description. Food codes and descriptions are part of USDA's Food and Nutrient Database for Dietary Studies (FNDDS) for foods and beverages reported in the What We Eat in America (WWEIA) component of the National Health and Nutrition Examination Survey (NHANES) (link to USDA's FNDDS website). Please clarify the information presented in this appendix and confirm whether this impacts the estimates of dietary exposure provided in your notice:

a. "milk chocolate candy, plain" should be 91705010
b. "chocolate, sweet or dark" should be 91705300
c. "fruit juice drink, with high vitamin C, plus calcium" should be 92582100
d. "fruit flavored drink, powdered, not reconstituted" should be 92900110
e. "fruit flavored drink, powdered, not reconstituted, diet" should be 92900200
f. "FUZE Slenderize fortified low-calorie fruit juice beverage" should be 95341000
g. "fruit juice, acai blend" should be 95342000
h. 91361020 should be "fruit sauce"
i. 91404000 should be "marmalade, all flavors"
j. 91708000 should be "fruit peeled, candied"
k. 91708010 should be "date candy"
l. 92804000 should be "Shirley temple"
m. 93404560 should be "sangria"
n. 93504100 should be "rum cooler"

Response: A review was made by the company that had performed the EDI calculation using the food codes from the GRAS Notice as compared to the current FNDDS. They identified the problem. The original excel file was created correctly; however, during reformatting a few food code lines were switched. The names of the foods in the GRAS Notice were correct, while the food codes were not. The desired foods are the ones listed in the FDA Chemistry review items 3. a. – g. The food names listed in h. – n. were not

intended to be included in the EDI. The food codes provided in the FDA letter for a. – g. are correct and have been changed. After changing the food code numbers analyses were performed and a revised report provided. It was determined that the original EDI calculations were correct, and the change does not impact the EDI. The revised report can be provided to the Agency upon request.

4. FDA responded without questions to a GRAS notice (GRN 000796) for the use of orange extract that contains ≥85% hesperidin in a variety of foods at levels up to 500 mg/serving. Please discuss how the intended use of glucosyl hesperidin and the probable cumulative dietary exposure to hesperidin compares to this previously notified use. For example, to what extent is the use of glucosyl hesperidin additive or substitutional for the use of the hesperidin-containing ingredient described in GRN 000796.

Response: The Notifier does not believe that the use of glucosyl hesperidin (GH) and hesperidin, as described in GRN 000796, will have an effect on cumulative dietary exposure. The first reason is that hesperidin has several intended uses that are not requested for GH, namely flavored milk and imitation milk drinks, dry powdered milk mixtures (not reconstituted), coconut beverages, cookies, cereal, cereal/granola/ nutrition bars, and table fats and vegetable oils. Therefore only hesperidin can be used in these foods. Second, the intended use of hesperidin is as a “source of bioflavonoids”. No other technical or physical effects were given. Conversely, GH will be used as a food ingredient to provide an antioxidant source that can function as a coloring adjunct (not a color, see Regulatory review item 2), flavor enhancer, and as a flavoring agent and adjuvant in the finished product. Third, because of the insolubility of hesperidin in water (about 2 mg/100 ml), the Notifier does not believe that hesperidin will be suitable for use in certain products and/or manufacturing processes. This was the original rationale for the development of GH. If used in an aqueous drink at 500 mg/serving (355 ml) it would make the concentration approximately 140 mg/100 ml, which would likely precipitate during processing or storage, unless the drink matrix is designed to increase the solubility. Whereas, the high solubility of GH could be easily used, but at a much higher cost. This is the fourth rational, namely GH is manufactured using the end product of the GRASed hesperidin product as a raw ingredient. When a purified preparation of hesperidin is used as the raw material for GH it requires enzymatic synthesis, re-purification, concentration and finished product handling. Therefore there would need to be a good rationale for choosing GH over hesperidin in a food product. If hesperidin provided the desired benefit, then GH would not likely be used. Conversely, if GH provided a cost-sensitive benefit to end product quality for the intended use it would be considered. Fifth, the use of both ingredients in a single product would not be practical because GH provides product quality benefits for which hesperidin is not intended. Considering the intended uses, technical and physio-chemical characteristic functions, and relative expense of these two ingredients, the Notifier believes that GH would only be used as a substitutional ingredient for hesperidin when specifically warranted.

5. On page 14 (Table 2-3), the notifier describes the analytical methodologies used to determine compliance with specifications that include methods established in Japan's Specifications and Standards for Food Additives, Japanese Pharmacopeia, Japan Industrial Standard, as well as “in-house” HPLC methodologies. Please confirm that all

methodologies used are validated for the determination of the respective analytes.

Response: The Notifier understands that the general methods established in the Japanese official compendiums, such as Japan's Specifications and Standards for Food Additives, Japanese Pharmacopeia and Japan Industrial Standard, are officially validated by the Japanese governmental laboratories, and are accepted in Japan as official methods for their respective analytes. Therefore, the Notifier has not validated these methods.

The following is the report for the validation of the "in-house HPLC method" for "Monoglucosyl hesperidin (MGH)" and "Total hesperidin":

1) Preparation of the test and the standard solutions:
Weigh accurately about 0.5 g of the dried test sample and dissolve it into purified water to make it accurately to the 100 mL solution. Measure accurately 4 mL of this solution and add it into the mobile phase to make accurately it to the 100 mL test solution.
Separately, weigh accurately about 0.05 g of dried MGH standard* and dissolve it into the mobile phase to make it accurately to the 250 mL standard solution.

2) HPLC determination:
Inject 10 µL of the test and standard solutions into HPLC under the following operating conditions. Determine the peak area of MGH and Hesperidin by the chromatogram.
The relative retention time of hesperidin against MGH is about 1.1.

[Operating conditions for HPLC]

Detector:	Ultraviolet absorption detector (Wave length: 280 nm)
Column:	CAPCELL PAK C18 UG120 Φ4.6 mm × L 250 mm (Shiseido Co., Ltd.) or the equivalent
Mobile phase:	Water/acetonitrile/acetic acid = 80/20/0.01
Flow rate:	About 0.75 mL/min (adjust the rate for the retention time of MGH to be 15 min)
Column temperature:	40°C

3) Calculation:
Based on the chromatograms of the test and standard solutions, calculate a) MGH and b) total hesperidin quantities using the following equation:

a) MGH (%) = (ATM / AS) × (WS / WT) × 100 / 250 × 25 × 100
b) Total hesperidin (%)
$= [(A_{TM} + A_{TH}) \times 0.790 / A_S] \times (W_S / W_T) \times 100 / 250 \times 25 \times 100$

A_{TM}: The peak area of MGH in test solution
A_{TH}: The peak area of Hesperidin in test solution
A_S: The peak area of MGH in standard solution
W_S: The amount of MGH standard
W_T: The amount of sample

$$0.790: \quad \frac{\text{Molecular weight of Hesperidin (610.56)}}{\text{Molecular weight of MGH (772.70)}}$$

* MGH standard
  Prepared in the Notifier, Hayashibara Co., Ltd. (Okayama, Japan)
  This standard is light yellow to yellowish brown powder with slight characteristic odor.
  Identification (1):
    Dissolve 5 mg of the substance to 10 mL of purified water and add 1 or 2 drops of ferric chloride TS1 to this solution. The solution turns into brown.
  Identification (2):
    Dissolve 0.01 g of the substance to 500 mL of purified water. The maximum absorption of this solution is identified at 280 – 286 nm.
  Loss on drying (following the “Appendix II: Physical Tests and Determinations” on the FCC):
    Not more than 6.0%
  Related substances:
    Accurately weigh about 0.1 g of the substance and dissolve into water/acetonitrile/acetic acid (80:20:0.01) to make 200 mL solution. This solution is used as test solution. Accurately measure 1 mL of test solution and dilute with water/acetonitrile/acetic acid (80:20:0.01) to make 50 mL solution. This solution is used as control solution.
    Inject 10 µL of the test and control solutions into HPLC and determine the peak areas by each chromatogram. The operating conditions are the same as described above and the measuring time is twice as long as the retention time of the main peak.
    The sum of other peak areas of test solution than the main peak area should not larger than the peak area of the main peak of control solution.

The conditions of the above “2) HPLC determination” are established in the “Enzymatically Modified Hesperidin” monograph of Japan's Specifications and Standards for Food Additives. Therefore, system suitability tests were performed on the HPLC analysis system used by the Notifier.

In the “Enzymatically Modified Hesperidin” monograph of Japan's Specifications and Standards for Food Additives, "confirmation of detectability", "system performance", and "system reproducibility" are required. However because the system suitability standards are not specified in the compendium, they were determined from the actual measurement data. Therefore, the Notifier determined the linearity, separation degree, and reproducibility.

<Analysis to establish the system suitability standards>

Weigh 0.02 g or 0.026 g of MGH (reagent grade) and 0.02 g of hesperidin (reagent grade), and dissolve with the mobile phase for HPLC to accurately make a 100 mL solution (Test solution). Dilute 10 times with the mobile phase (Dilution). Test solution) and Dilution were analyzed by HPLC under the conditions above using the column,

CAPCELL PAK C18 UG120 Φ4.6 mm × L 250 mm (Shiseido Co., Ltd.), Lot Nos. AOAD21527 (used) and AOAD28771 (new).

Colum: Lot Nos. AOAD21527 (used)
Sample weight used / MGH : Hes = 0.026 g : 0.02 g (undiluted solution)

No.	MGH			Hes			Separation degree
	Peak area	Average	FWHM*	Peak area	Average	FWHM*	
1	4934993	4990656	0.313	4411985	4459985	0.353	2.42
2	4971086		0.314	4437671		0.353	2.42
3	4984442		0.313	4470389		0.353	2.42
4	4996974		0.313	4457976		0.352	2.43
5	5013922		0.313	4483919		0.353	2.42
6	5042516		0.312	4497970		0.351	2.43
CV†	0.74%			0.70%			

Colum: Lot Nos. AOAD28771 (unused)
Sample weight used / MGH : Hes = 0.02 g : 0.02 g (undiluted solution)

No.	MGH			Hes			Separation degree
	Peak area	Average	FWHM*	Peak area	Average	FWHM*	
1	4158283	4207433	0.296	4568243	4619457	0.339	2.63
2	4177226		0.296	4591992		0.339	2.63
3	4181507		0.295	4595519		0.338	2.63
4	4191310		0.297	4605735		0.340	2.63
5	4262941		0.295	4677700		0.338	2.64
6	4273328		0.296	4677551		0.338	2.63
CV†	1.2%			1.0%			

Colum: Lot Nos. AOAD28771 (unused)
Sample weight used / MGH : Hes = 0.02 g : 0.02 g (10 times dilution)

No.	MGH		Hes	
	Peak area	Average	Peak area	Average
1	407160	407714	446888	447153
2	406578		445710	
3	409405		448860	
Linearity		10.3%		10.3%

* FWHM: Full width at half maximum, CV: Coefficient of variation

As a worst case, a column which had been used in the QC section for about 2 years were also used for the analysis. As the results above, the standards were established as below:

Confirmation of detectability: The peak area ratio of 10 times dilution to the undiluted solution on MGH and Hes is 7 – 13.
System performance: Separation degree: Not less than 2.
System reproducibility: CV: Not more than 1%.

<System suitability test>

Weigh 0.02 g of MGH (reagent grade) and hesperidin (reagent grade), dissolve with 4 mL of 0.05 mol/L NaOH solution, and dilute with the mobile phase for HPLC to accurately make each 100 mL solution. Dilute 10 times with the mobile phase. The undiluted solution and dilution were analyzed by HPLC under the conditions described above. The test on the undiluted solution and dilution were repeated 6 and 3 times, respectively.

The results are shown below

Sample: Undiluted solution

No.	MGH			Hes			Separation degree
	Peak area	Average	FWHM	Peak area	Average	FWHM	
1	3482760	3460234	0.298	4228442	4227545	0.335	2.39
2	3471370		0.298	4230954		0.335	2.39
3	3458774		0.298	4227234		0.335	2.39
4	3451190		0.298	4222170		0.335	2.40
5	3446396		0.299	4225341		0.336	2.39
6	3450913		0.299	4231126		0.336	2.39
CV	0.4%			0.1%			

Sample: 10 times dilution

No.	MGH		Hes	
	Peak area	Average	Peak area	Average
1	337275	331921	402884	399643
2	324520		396428	
3	333969		399616	
Linearity		9.6%		9.5%

The results are shown below:

Confirmation of detectability: The peak area ratio of 10 times dilution to the undiluted solution on MGH and Hes was 9.59% and 9.45%, respectively.

System performance: The separation degree was 2.39 – 2.40.

System reproducibility: The CV of MGH and Hes was 0.4% and 0.1%, respectively.

<Conclusion>

The HPLC system used for the analysis of MGH and Total hesperidin met the standards established above.

Toxicology review

1. Please provide data missing from your published rat subchronic study of glucosyl hesperidin (Matsumoto et al., 2019), particularly those that pertain to excluding liver and testicular toxicity. We suggest you provide:

    a. Summary tables for each dose group including body weights, all organ weights (absolute and relative to body weight), all hematology, clinical chemistry and urinalysis measurements

    b. Tabular summary of relevant gross lesions and histopathology observations (e.g. testicular and liver findings discussed in the text of the publication).

Response: Please see the summary Tables from the 13-Week Oral Toxicity (Feeding) Study in the Rat. The attachment is labeled as "13-week Rat Summary Tables". The Tables include the following data by sex: body weight, organ weight, organ/body weight ratios, hematology, clinical chemistry, urinalysis, macroscopic findings (all necropsies), pathology summary reports.

2. Please discuss how the higher bioavailability of glucosyl hesperidin relates to the dietary exposure and also to the no-effect levels from clinical and rodent studies of hesperidin and other flavonoids to support the safety of glucosyl hesperidin.

Response: The water solubility of hesperidin is about 0.002 g/100mL, and GH is slight less than 200 g/100 mL. In the study of Yamada et al. (2006a, published) the bioavailability of an aqueous solution of monoglucosyl hesperidin in rats (MGH; the major component of GH) was compared to hesperidin in water. The animals were fasted overnight before treatment. The plasma concentration of MGH (hesperetin) was demonstrated to be approximately 3.7 times that of hesperidin. In a cross-over study by Nielsen et al. (2006) reported in the GRAS Notice, subjects consumed orange juice (300 mL) with natural hesperidin and a second orange drink with 3 times the amount of hesperidin as the first. Values of the AUC of total plasma hesperetin was approximately 3.6 times greater than the normal juice (1.16 ± 0.52 mmol/(L·h versus 4.16 ± 1.5 mmol/(L·h)). Additionally in the same paper by Nielsen and co-workers a third sample was given the subjects in which the orange juice was enzymatically treated to form hesperitin-7-glucoside, which is about 30 times more soluble than hesperidin. In this instance the plasma hesperetin AUC was increased about 3 times (3.45 ± 1.27 mmol/ (L·h)) than that of the untreated natural orange juice. This suggesting that the same increase in bioavailability as GH can be obtained from either a larger portion of orange juice or a hesperidin-based substance with a solubility much lower than GH. It also indicates that there is a limit to the bioavailability as related to solubility.

Further, in another rat study similar to the one above, the same dose equivalent of hesperidin and MGH as given above was administered in a 0.2% aqueous solution of sodium carboxymethyl cellulose (CMC-Na) in 2 mL to fasted rats (Mitsuzumi et al., 2006, unpublished). CMC-Na was used to emulsify hesperidin. It is believed that this is a similar situation to hesperidin being consumed in a normal food matrix in the diet.

When CMC-Na was used, the absorption pattern, absorption rate, pattern of distribution, and pattern of excretion of hesperidin and MGH were essentially equal. However, the addition of CMC-Na to GH did not further increase the plasma concentration. It is thought that the normal consumption of hesperidin in the diet would predominantly be mixed in a food matrix, and would act in a similar manner to the added CMC-Na this experiment. Similar results have been observed in recent studies for pharmaceutical uses where 2-hydroxypropyl-beta-cyclodextrin (higher solubility than α, β, or γ cyclodextrin) and chitooligosaccharide, respectively to increase solubility (Majumdar and Srirangam, 2009; Cao et al., 2017; Rajabi, 2019). Conversely, in one of the few studies actually examining the intake of flavanones in orange juice with a food (full-fat natural yoghurt) there was reduced excretion of predominantly hesperidin (flavanone) metabolites by about 6.7 times (Roowi, et al., 2009). Suggesting the uptake of hesperidin can also be inhibited by certain foods.

These data indicate that when hesperidin or GH are consumed in an aqueous solution with a food matrix that can act as a solubilizer or suspension agent it may increase the bioavailability of hesperidin to that of GH, but does not further increase the GH bioavailability. It is believed that much of the hesperidin consumed in a normal diet would be in conjunction with a food matrix, which may or may not interfere with uptake.

Concerning the safety of GH and no-effect levels, the pivotal 90-day oral consumption study reported by Matsumoto et al. (2019) was performed with GH. This would result in a greater systemic exposure (3.7 times) than if hesperidin had been administered. The NOEL (NOAEL; the original report stated, “[N]o adverse effects were observed.”) of GH was calculated as 3,256 mg/kg-bw/day (mean female and males groups), which is equal to a safety factor of greater than 100 (total population group, consumers only, 90th percentile). However, if the relative exposure to hesperidin (hesperetin) is 3.7 times greater than if an equal amount of hesperidin was administered it could be argued that the actual NOAEL would be 3.7 times greater. The 90-day oral consumption study demonstrates that even if GH is absorbed at a greater concentration, it is still safe. This safety factor does include both the GH for intended use and naturally occurring hesperidin in the human diet. Also the molecular weight of GH is about 20% less than for an equal amount of hesperidin, which would reduce the relative total amount of hesperidin that is taken into the body in the 500 mg dose.

As was demonstrated in several studies in rats and humans, GH is only absorbed into the body as hesperetin in an identical manner as natural hesperidin. In the 96-week subchronic carcinogenic study of methyl hesperidin in mice that was used to support the safety of GH, it provides a NOAEL of 7,500 mg/kg bw/day (Kurata et al., 1990). This was used as the primary pivotal study for GRN 000796. It was not stated in the GRN 000796 that methyl hesperidin has solubility approximately 5,000 times that of hesperidin. Assuming that the absorption of mice was similar to that of rats, this would mean that the uptake of the associated hesperidin into the body of the mice would be much greater than if hesperidin was consumed. The original comparison of this mouse study to the GH EDI was greater than 400 fold for the total population group, consumers only, 90th percentile. If there were a plasma concentration differential of hesperetin for the consumption of

hesperidin as compared to methyl hesperidin then this would increase, as discussed with the GH NOAEL, the relative NOAEL for the study, and therefore the safety factor. Flavonoids are classified into 12 subclasses; however there are essentially 6 of these subclasses that are of human dietary importance. These include flavonols, flavan-3-ols, flavones, flavanones and anthocyanindins, and isoflavones. Each of these subgroups contains 3 to 6 major chemicals. As mentioned previously hesperidin is a flavanone. According to the NHANES estimated flavonoid intake in US adults average between 200 and 250 mg/day. The approximately percentage of each subclass it 80% flavan-3-ols, 8% flavonols, 6% flavanones, 5% anthocyanindins, and $\leq$ 1% isoflavones and flavones. The flavanone consumption in Europe is about 5%. This calculates to about 15.0 mg/day consumption of hesperidin using the higher mean of 250 mg/day average intake. That calculates to 0.25 mg/kg/day in a 60 kg person. From the NOAEL of 7,500 mg/kg/day of methyl hesperidin to the NOAEL of 3,256 mg of GH/kg-bw/day it would appear that there is sufficient safety to include GH in the daily consumption of flavanones.

The other main flavanone that is found in foods containing hesperidin is naringin. In a 6-month chronic toxicity study in which rats were given up to 1,250 mg/kg naringin by oral gavage, the NOAEL was calculated at 1,250 mg/kg/day (Li et al., 2014). Previous to this study the same authors reported an acute and a 13-week subchronic toxicity study. The acute study used a dose of 16 g/kg and the 13-week study the 1,250 mg/kg/day that was used in the 6-month study. Again the authors reported a NOAEL of 1,250 mg/kg/day (Li et al., 2013). These data show that the safety of another flavanone that is usually found in many of the same foods as hesperidin has a relatively high NOAEL. This would suggest that the increase of hesperidin would not likely product any safety issues if consumed in the diet with foods that naturally contain hesperidin and naringin.

These references in the information above were not in the original GRAS Notice 000901.

Roowi S, Mullen W, Edwards CA, Crozier A. 2009. Yoghurt impacts on the excretion of phenolic acids derived from colonic breakdown of orange juice flavanones in humans. Mol Nutru Food Res 53 Suppl 1:S68-75.

Cao R, Zhao Y, Zhou Z, Zhao X. 2017. Enhancement of the water solubility and antioxicant activity of hesperidin by chitooligosaccharide. J Sci Food Agri 98:2422-2427.

Majumdar S, Srirangam R. 2009. Solubility, stability, physiochemical characteristics and *in vivo* ocular tissue permeability of hesperidin: a natural bioflavonoid. Pharm Res 26:1217-1225.

Li P, Wang S, Guan X, Liu B, Wang Y, Xu K, Peng W, Su W, Zhang K. 2013. Acute and 13 weeks subchronic toxicological evaluation of Naringin in Sprague-Dawley rats. Food Chem Toxicol 60:1-9.

Li P, Wang S, Guan X, Cen X, Hu C, Peng W, Wang Y Su W. 2014. Six months chronic toxicological evaluation of naringin in Sprague-Dawley rats. Food Chem Toxicol 66:65-75.

3. On page 51, the notifier states that hesperidin may not enter the enterohepatic cycle in humans, a contrast with rats. Please discuss if this difference impacts your safety assessment.

Response: The comment that hesperidin may not enter the enterohepatic cycle in humans was simply a reflection of the Notifier's inability to find specific information on this subject in the literature. Hesperidin is unlikely to be available for biliary excretion as it undergoes extensive hydrolysis before absorption in the lower intestine. The major hydrolysis product, hesperetin, would not be expected to undergo biliary excretion in

humans as its molecular weight is to low, but conjugated derivatives may be excreted in the bile.

In a recent review of citrus flavanone metabolism Stevens and co-workers provide data from a number of human studies where citrus products, primarily orange juice containing hesperidin, were consumed and the metabolites identified from the plasma, urine and/or feces (Stevens et al., 2019). It was shown that hesperetin undergoes extensive metabolism principally by the microbiota in the colon. As observed in other studies there is a wide subject variation in bioavailability and in the types of metabolites produced. This variation of metabolites is thought to result from interindividual differences in the microbiome. However, results of studies in rats have reported that hesperidin metabolites undergo enterohepatic cycling in this species. No adverse reactions have been reported as a result of the recycling of these hesperidin (hesperetin) metabolites in rats. It is reported that these citrus flavanones and their metabolites likely contribute to increased gastrointestinal function and health (Stevens et al., 2019).

Referring back to the original question, if there is little or no enterohepatic cycling of hesperidin metabolites in humans these metabolites would be excreted more rapidly and have less effect. Conversely, if there is enterohepatic cycling in humans it seems unlikely that these metabolites are of safety concern. This conclusion is supported by the following: no adverse effects were observed in the 96-week mouse oral safety study using methyl hesperidin (NOAEL 7,500 mg/kg/day), in the 13-week rat study of GH (NOEL 3,256 mg/kg/day), or in several human studies using GH. As noted before, both GH and methyl hesperidin, like hesperidin, are assimilated into the body as hesperetin and therefore should be metabolized in essentially the identical manner. Therefore, whether enterhepatic cycling occurs in humans or not, it is not believed that this would have an effect on the safety of consumption of GH.

Reference not in GRN 00910.

Stevens Y, Van Rymenant E, Grootaert C, Van Camp J, Possemiers S, Masclee, A Jonkers D. 2019. The intestinal face of citrus flavanones and their effects on gastrointestinal health. Nutrients 11:1464-1480.

4. Please discuss why some of the pharmacological effects mentioned in the human and animal studies (such as lowering blood pressure and changing blood lipid variables) are not a safety concern for normotensive or otherwise healthy consumers.

Response: In reviewing the raw data from several of the animal and human studies summarized in the GRN 000901, it is seen that glucosyl hesperidin (GH) does not cause safety concerns to normotensive or otherwise health subjects. The following studies will provide a summary of only the healthy/normotensive animals or humans, unless the data of the other subjects is instructive for comparative purposes.

Animal studies

In the sub-chronic 13-week oral toxicity study by Matsumoto et al., GH was mixed with a standard diet and feed to healthy female and male HanRcc:WIST(SPF) rats (Matsumoto et al., 2019). The 4 groups consisted of 10 female and 10 male rats with groups receiving 0, 4,500, 15,000 or 50,000 ppm/day. No statistical differences were seen in total

cholesterol (Total-C), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), or TG (TG) between the 0 ppm, and any of the treatment groups for either gender. This suggests that consumption of high concentrations of GH do not lower serum lipid variables.

Naiki et al. had 21 female Japanese white rabbits (7/group) consume 150 or 4,500 mg/day of α-glucosylhesperidin in water for 24 weeks. Comparison of the control with the 4,500 mg/day group did show that the mean aortic blood pressure was elevated ($p < 0.05$) at the end of the study, but was not out of the normal range for these rabbits. This indicates that normotensive rabbits did not decrease mean aortic blood pressure. This study was performed using another company's preparation of GH.

Twelve (12) male normotensive Wistar-Kyoto (WKY) rats were fed either a normal diet or the diet supplemented 30 mg/kg/d GHES (GH) for 25 weeks (Ohtsuki et al., 2002). Heart rate and systolic blood pressure was measured each week. The heart rate both groups decreased at similar rate from 5 to 30 weeks of age. During the 25 weeks there were 3 scattered samples that the heart rate of the GH group was either significantly ($p < 0.05$) higher (1) or lower (2), than the control group. The authors stated that, ".... a significant difference from the control group was scarcely observed throughout the experimental period." Systolic blood pressure of the GH group was significantly greater ($p < 0.05$ & $p < 0.01$) than controls 3 times during the first 5 weeks of treatment. Following that there were no significant differences. The authors concluded that, "[I]n the present study, the antihypertensive effects from the long-term administration of .... GHES (GH) were observed in the hypertensive rats but not the normotensive rats. The data suggests that over 25 weeks of consumption there was no consistent difference in animals receiving the GH or control diet.

As a follow-up to the last study, Ohtsuki and co-workers used 12 normotensive WKY rats and fed 6 animals a standard diet, with another group of 6 animals having 30 mg/kg/day GHES (GH) added to the feed for 25 weeks (Ohtsuki et al., 2003). One of the intended purposes of this study was to identify any changes in serum lipid composition. Total-C, LDL-C, TG, Free glycerol, free fatty acid, HDL-C, and HDL-C/Total-C (%) were measured at the beginning and end of consumption. There were no significant differences between the control group and GH treated group after 25 weeks of consumption.
GH (50 mg/kg/d) in a standard diet was administered to 6 WKY rats and the standard diet was given to a second group of 6 rats for 8 weeks (Yamamoto et al., 2008a). Systolic blood pressure was measured weekly. No significant differences in systolic blood pressure were noted in the normotensive rats from either the control or treatment group. Further, there were no differences between heart rate in either group at the end of the experimental period. Once again suggesting that consumption of GH over an extended time period does not affect heart rate or systolic blood pressure.

A single dose study in which 50 mg/kg of GH or a placebo control was administered to WKY rats examined the change in systolic blood pressure over 24 hours (Yamamoto et al., 2008b). There were slight % changes of (approximately $\pm$ 2%) over the 24-hour period. However, the profile of the control was essentially the same as the treatment group, and no significance was observed between the two groups, suggesting that GH has no effect on normotensive rats.

Matsumoto and co-workers (2019) reported on a 13-week oral feeding study where 10 female and 10 male rats were fed GH at 4,500, 15,000, 50,000 ppm, or control in their standard diet (Matsumoto et al., 2019). Analysis of Total-C, HDL-C, LDL-C and TG in serum was performed. No clinically or statistically significant differences were seen after 13 weeks of consumption of both female and male dose groups in lipid-related values.

Human studies

A study was performed on normal and hyperlipidemic subjects. The normal subjects were classified as having Total-C <230 mg/dL and TG <150 mg/dL. The test substance was a dose of either 100 or 500 mg/day in a table formulation for 6 weeks (Miwa et al., 2004). The normal subjects were divided into the two treatment groups. At 6 weeks Total-C, LDL-C, HDL-C, TG, Apo B, Apo C-II, Apo E, LDL-C/Apo B, and LDL-C/Apo B. The values of each variable were tested at 0, 2 and 6 weeks. The only value in the normal group that was significantly different ($p < 0.05$) as compared to week 0 was the Apo E in the 500 mg group at 2 weeks. It became non-significant at the end of treatment. These data suggest that serum lipid values do not change in individuals with normal Total-C and TG.
In a second study by Miwa and co-workers they examined lowering of TG (Miwa et al., 2005). The normal group of 6 subjects was identified as persons having initial fasting TG values of <110 mg/dL. The subjects consumed 500 mg of GH in two tablets daily for 24 weeks. Subjects had no significant differences in TG and HDL-C values at any time during treatment. Remnant-like particle cholesterol (RLP-C) was significantly increased from baseline ($p < 0.05$) at 8, 12, 16 and 20 weeks, but not at 24 weeks of treatment. Additionally it was within the normal range of RLP-C. Total-C and LDL-C had a slow reduction in their values ($p < 0.05$) at weeks 16, 20 and 24. Both mean Total-C and LDL-C values at week 0 were slightly greater than the normal reference range. Total-C ended in the normal range; whereas, while LDL-C was significantly reduced during consumption it remained slightly elevated. Serum concentrations of 5 apolipoproteins were examined. Apo B, Apo C-II, and Apo E had no significant changes in values over time. The values of Apo A-I, and Apo C-III had a significant increase ($p < 0.05$) at week 4. The very low-density lipoprotein (VLDL)/low density lipoprotein (LDL; VLDL/LDL is also known as small particle LDL ratio below) value was calculated at 0, 12 and 24 weeks of treatment, and there was no significance. While a few values in the normal <110 mg/dL TG group increased or decreased over the 24 weeks, most were within normal ranges and suggest that consumption of 500 mg/day for 24 weeks in subjects with normal TG is safe for human consumption.

In the study by Nakagawa and co-workers they investigated the effect of GH on hyperlipidemic and normal subjects using 500 mg/GH per day for 12 weeks (Nakagawa et al., 2008). Additionally a second experiment was performed where GH was given at 3 times the normal 500 mg/day dose (1,500 mg GH/day) for 4 weeks. The 28 subjects included 12 females and 16 males classified as mild hyperlipidemic (n=13) or normolipidemic (n=15) individuals. The TG concentration of the normal subjects did not change throughout the 4-week treatment. For analysis of the other lipid-associated variables the mild hyperlipidemic and normal subjects were not separated for analysis. However, there were no statistical differences in the values from week one to 4 weeks of treatment. The variables included Total-C, RPL-C, VLDL-C, HDL-C, LDL-C, free fatty acids and phospholipids. The only exception was the mean LDL particle size changed from 266.3 ± 0.9 angstroms to 267.8 ± 0.08 angstroms ($p < 0.01$). These data strongly

suggest that daily consumption of GH for 4 consecutive weeks do not lower the TG values in normal subjects. Further when normal and mildly hyperlipidemic subjects are groups together, the other blood lipid variables do not decrease.

In another study with two experiments there was a 12-week treatment of subjects with elevated TG with either 500 mg/day of GH in a 500 mL beverage or placebo (Tanaka et al., 2010). The second experiment was designed to look for safety using a dose of about 1,500 mg GH/day for 4 weeks. Thirty-four (34; see summary in Part 6) healthy subjects consisted of 18 individuals with normal TG, and 16 with TG levels of 120-200 mg/dL. The lipid variables evaluated were TG, RLP-C and phospholipids. After 4 weeks of receiving 1,500 mg GH/day there were no changes in the lipid variable values of the control group, whereas the other groups values significantly decreased (at least $p < 0.05$).

A green tea beverage containing GH or placebo was given to subject to see the effect on various blood lipid variables as in the human studies provided above (Yuasa et al., 2005). Subjects in the 12-week open study included 10 healthy individuals (6 females, 4 males) with TG values in the normal range. They were given 500 mg GH/daily for 12 weeks. There was a 4-week post consumption sampling time. The lipid-related analyses included TG, Total-C, LDL-C, HDL-C, VLDL-C, RPL-C, small particle LDL ratio, mean LDL particle size, phospholipids, Apo A-I, Apo A-II, Apo B, Apo C-II, Apo C-III, Apo E, free fatty acids and ketone bodies. After 12 weeks of consumption none of the lipid variables in the normal subjects had changed from baseline, expect two. The mean small particle size was significantly greater than baseline ($p < 0.05$ to 0.01) at 4, 6, 12 and 16 weeks (values for week 0–16 were 265±3, 267±3, 270±4, 269±3 and 268±3 angstroms). The other significant variable was small particle LDL ratio. It was significantly reduced ($p < 0.05$ to 0.01) at all sampling times (values for week 0-16 were 0.011±0.08, 0.006±0.05, 0.003±0.06, 0.003±0.06 and 0.004±0.04%). These significant differences are generally considered a positive event. The authors concluded, “this beverage is unlikely to reduce TG level to levels lower than the normal range.”

The final experiment included 9 normolipidemic subjects who were given about 1,500 mg GH/day for 4 weeks with a 2-week post consumption sampling. Comparison of week 0 with week 4 values of all the same lipid variables in the experiment as given above in the first experiment showed no statistical differences after a 4-week treatment of 1,500 mg GH/day, expect one. There was a significant increase ($p < 0.01$) in the mean LDL particle size (week 0 269±2 and week 4 271±1 angstroms). This difference continued for the 2-week non-consumption period. Any increase in this variable would be considered a positive event. The authors stated, “no problematic change was noted in any lipid-related parameter”?

In conclusion, in all animal and human studies where large amounts of GH were administered (50,000 ppm per day in rats for 13-weeks, and in humans at 500 mg/day for 12 weeks or at 1,500 mg GH/day for 4 weeks, no reductions were seen that would suggest a problem for normolipidemic or otherwise individuals consuming at least the 500 mg GH/day that was requested in this GRAS Notice. In the Miwa et al., 2005 paper no statistical significance was observed in the mean LDL particle size (VLDL/LDL), while it was in the Nakagawa et al., 2008 and Yuasa et al., 2010 publication. However, it is not believed that the changes seen in these variables are to be considered a negative effect.

5. In the notice, the notifier cites and discusses several studies on glucosyl hesperidin published in Japanese. Please provide copies of the original publications in Japanese as well as English translations verified to be complete and accurate.

Response: The Japanese publications are provided in the attachment file "Translation Publications". They are provided by the first author's last name and date of the publication as given in the references. There is also an attached file labeled "Translation verification", which contains the requested statements of verification for accuracy and completeness.

Regulatory review

1. On page 6, the notifier states that the notice is submitted in accordance with 21 CFR 170.225 Part 1. We note that 21 CFR 170.225(1) states that in Part 1 of a GRAS notice the notifier must inform us that the notice is submitted in accordance with Subpart E. Please provided a correct statement.

Response: Vanguard Regulatory Services, Inc., on behalf of Hayashibara Co., Ltd. (Hayashibara), of Okayama, Japan, submits this GRAS notice of Glucosyl Hesperidin in accordance with 21 CFR §170 subpart E.

2. On page 6, the notifier states that one of the intended uses of glucosyl hesperidin is as a coloring adjunct. On page 30 (Table 2-12), the notifier lists technical effects for which glucosyl hesperidin may be added to foods. According to entry (4) in Table 2-12, glucosyl hesperidin maybe added to food as a color and coloring adjunct. We note that the use of glucosyl hesperidin as a color additive would require premarket approval by FDA. Please provide a statement that glucosyl hesperidin is not intended for use as a color additive.

Response: On page 30, Table 2-12 the table provides the Technical effects of glucosyl hesperidin (GH) from 21CFR §170.3 (o). In item (4) it is "Colors and coloring adjuncts". There is a footnote stating that GH is not a color. This was also stated on page 6, Part 1.5, first paragraph.

3. On page 93, the notifier states that the Appendices to the notice include "...a report (2009) and subsequent updated letter (2019) by an independent GRAS Panel...." We note that only the updated letter dated December 1, 2019 was included in the notice. Please provide the 2009 report for the completeness of our records.

Response: The 2009 GRAS Panel Report is attached as a separate file named "GRAS Panel Report 2009."

Document No. IER01-76-022
May 25th, 2020

Organo Corporation
Functional Materials Department

- **AMBERLITE™ IRA96SB**
- **AMBERLYST™ 16WET**

We certify that these ion exchange resin products meet the "Ion Exchange Resin" monograph in Japan's Specifications and Standards for Food Additives of the Food Sanitation Law.

The above information relates specifically to the product reviewed. We recommend customers make their own determination of resin suitability for their particular intended use(s). We believe this information is reliable as of the date of this letter.

ORGANO CORPORATION
1-2-8 Shinsuna, Koto-ku, Tokyo 136-8631
TEL:03-5635-5100 FAX:03-3699-7030

BODY WEIGHTS (GRAM) SUMMARY
MALES

TREATMENT			GROUP 1 0 PPM	GROUP 2 4500 PPM	GROUP 3 15000 PPM	GROUP 4 50000 PPM
DAY 1		MEAN	191.8	187.6	187.1	185.0
WEEK 1		ST.DEV.	7.1	3.8	8.8	8.0
		N	10	10	10	10
DAY 8		MEAN	237.8	234.1	230.7	227.8 *
WEEK 2		ST.DEV.	7.2	6.1	10.1	10.2
		N	10	10	10	10
DAY 15		MEAN	278.0	274.9	270.5	267.4
WEEK 3		ST.DEV.	9.0	10.4	13.4	12.2
		N	10	10	10	10
DAY 22		MEAN	302.9	302.8	295.7	293.9
WEEK 4		ST.DEV.	10.5	15.8	17.2	16.1
		N	10	10	10	10
DAY 29		MEAN	321.1	324.7	317.1	313.6
WEEK 5		ST.DEV.	11.5	23.9	19.9	20.8
		N	10	10	10	10
DAY 36		MEAN	342.5	346.8	338.4	334.6
WEEK 6		ST.DEV.	14.4	28.0	20.9	24.1
		N	10	10	10	10
DAY 43		MEAN	359.5	361.0	351.2	347.5
WEEK 7		ST.DEV.	14.5	29.9	21.9	26.4
		N	10	10	10	10
DAY 50		MEAN	374.9	375.4	365.2	361.8
WEEK 8		ST.DEV.	15.5	31.7	23.9	28.9
		N	10	10	10	10
DAY 57		MEAN	384.9	393.5	378.4	377.3
WEEK 9		ST.DEV.	16.6	32.4	26.5	32.2
		N	10	10	10	10
DAY 64		MEAN	399.2	404.1	386.6	385.8
WEEK 10		ST.DEV.	18.9	34.6	28.8	34.1
		N	10	10	10	10
DAY 71		MEAN	410.8	413.9	397.2	395.6
WEEK 11		ST.DEV.	18.5	35.8	30.6	35.2
		N	10	10	10	10
DAY 78		MEAN	422.7	422.7	416.4	403.5
WEEK 12		ST.DEV.	19.6	36.0	28.7	36.4
		N	10	10	10	10
DAY 85		MEAN	428.8	429.1	413.3	410.5
WEEK 13		ST.DEV.	22.1	37.9	34.5	38.3
		N	10	10	10	10
DAY 91		MEAN	435.9	437.1	419.7	416.6
WEEK 13		ST.DEV.	21.5	38.2	35.2	40.7
		N	10	10	10	10

* / ** : Dunnett-Test based on pooled variance significant at 5% (*) or 1% (**) level

BODY WEIGHTS (GRAM) SUMMARY
FEMALES

TREATMENT			GROUP 1 0 PPM	GROUP 2 4500 PPM	GROUP 3 15000 PPM	GROUP 4 50000 PPM
DAY 1		MEAN	144.0	143.8	147.3	143.4
WEEK 1		ST.DEV.	5.3	5.7	4.8	5.7
		N	10	10	7	10
DAY 8		MEAN	166.0	165.5	166.7	162.4
WEEK 2		ST.DEV.	4.7	6.0	4.5	7.6
		N	10	10	7	10
DAY 15		MEAN	184.2	185.9	187.4	181.4
WEEK 3		ST.DEV.	5.7	8.4	2.5	10.4
		N	10	10	7	10
DAY 22		MEAN	196.4	197.3	198.6	192.3
WEEK 4		ST.DEV.	5.1	11.5	5.9	8.9
		N	10	10	7	10
DAY 29		MEAN	207.9	210.3	215.1	205.9
WEEK 5		ST.DEV.	7.9	10.3	6.0	11.5
		N	10	10	7	10
DAY 36		MEAN	218.3	222.4	224.7	217.3
WEEK 6		ST.DEV.	8.0	11.2	5.7	11.4
		N	10	10	7	10
DAY 43		MEAN	225.1	230.6	233.2	223.6
WEEK 7		ST.DEV.	8.4	12.7	6.7	11.1
		N	10	10	7	10
DAY 50		MEAN	229.3	235.3	240.1	231.7
WEEK 8		ST.DEV.	8.6	13.8	9.1	9.8
		N	10	10	7	10
DAY 57		MEAN	235.3	241.7	248.1	238.7
WEEK 9		ST.DEV.	11.1	12.9	9.5	11.7
		N	10	10	7	10
DAY 64		MEAN	240.4	248.7	252.2	240.5
WEEK 10		ST.DEV.	10.5	15.8	7.7	12.7
		N	10	10	7	10
DAY 71		MEAN	242.6	252.0	255.0	243.8
WEEK 11		ST.DEV.	11.2	15.4	7.0	13.3
		N	10	10	7	10
DAY 78		MEAN	243.9	253.9	257.9	248.8
WEEK 12		ST.DEV.	12.1	18.3	7.7	13.7
		N	10	10	7	10
DAY 85		MEAN	248.3	254.5	263.0	248.3
WEEK 13		ST.DEV.	11.7	17.2	10.9	15.0
		N	10	10	7	10
DAY 91		MEAN	251.7	260.3	266.0	252.5
WEEK 13		ST.DEV.	11.1	16.3	8.4	14.7
		N	10	10	7	10

* / ** : Dunnett-Test based on pooled variance significant at 5% (*) or 1% (**) level

ORGAN WEIGHTS (GRAM) SUMMARY
AFTER 13 WEEKS
MALES

		GROUP 1 0 PPM	GROUP 2 4500 PPM	GROUP 3 15000 PPM	GROUP 4 50000 PPM
BODY W.	MEAN	408.50	410.84	391.61	386.40
	ST.DEV.	24.21	38.04	33.44	40.52
	N	10	10	10	10
BRAIN	MEAN	2.05	2.07	2.04	2.04
	ST.DEV.	0.08	0.10	0.06	0.08
	N	10	10	10	10
HEART	MEAN	1.122	1.088	1.084	1.085
	ST.DEV.	0.077	0.115	0.078	0.108
	N	10	10	10	10
LIVER	MEAN	9.75	9.69	9.28	9.15
	ST.DEV.	0.73	0.96	0.95	1.01
	N	10	10	10	10
THYMUS	MEAN	0.31	0.31	0.33	0.33
	ST.DEV.	0.05	0.05	0.05	0.07
	N	10	10	10	10
KIDNEYS	MEAN	2.22	2.13	2.08	2.14
	ST.DEV.	0.21	0.14	0.21	0.19
	N	10	10	10	10
ADRENALS	MEAN	0.069	0.061	0.063	0.063
	ST.DEV.	0.011	0.005	0.007	0.010
	N	10	10	10	10
SPLEEN	MEAN	0.731	0.788	0.789	0.724
	ST.DEV.	0.130	0.101	0.101	0.125
	N	10	10	10	10
TESTES	MEAN	4.18	3.80 *	3.92	3.80 *
	ST.DEV.	0.34	0.20	0.28	0.36
	N	10	10	10	10
EPIDIDYMIDES	MEAN	1.542	1.443	1.455	1.435
	ST.DEV.	0.160	0.119	0.156	0.153
	N	10	10	10	10

*/**; Dunnett-test based on pooled variance sig. at 5% or 1% level.

ORGAN/BODY WEIGHT RATIOS SUMMARY
AFTER 13 WEEKS
MALES

		GROUP 1 0 PPM	GROUP 2 4500 PPM	GROUP 3 15000 PPM	GROUP 4 50000 PPM
BODY W.	MEAN	408.50	410.84	391.61	386.40
(GRAM)	ST.DEV.	24.21	38.04	33.44	40.52
	N	10	10	10	10
BRAIN	MEAN	0.50	0.51	0.52	0.53
(%)	ST.DEV.	0.04	0.05	0.04	0.04
	N	10	10	10	10
HEART	MEAN	0.275	0.265	0.278	0.282
(%)	ST.DEV.	0.018	0.013	0.017	0.024
	N	10	10	10	10
LIVER	MEAN	2.39	2.36	2.37	2.37
(%)	ST.DEV.	0.14	0.16	0.16	0.13
	N	10	10	10	10
THYMUS	MEAN	0.08	0.08	0.08	0.09
(%)	ST.DEV.	0.01	0.02	0.01	0.01
	N	10	10	10	10
KIDNEYS	MEAN	0.54	0.52	0.53	0.56
(%)	ST.DEV.	0.05	0.04	0.05	0.05
	N	10	10	10	10
ADRENALS	MEAN	0.017	0.015	0.016	0.016
(%)	ST.DEV.	0.003	0.002	0.002	0.003
	N	10	10	10	10
SPLEEN	MEAN	0.179	0.192	0.201	0.188
(%)	ST.DEV.	0.030	0.024	0.019	0.027
	N	10	10	10	10
TESTES	MEAN	1.02	0.93	1.00	0.99
(%)	ST.DEV.	0.07	0.08	0.07	0.12
	N	10	10	10	10
EPIDIDYMIDES	MEAN	0.379	0.353	0.373	0.374
(%)	ST.DEV.	0.044	0.031	0.040	0.043
	N	10	10	10	10

*/**: Dunnett-test based on pooled variance sig. at 5% or 1% level.

ORGAN WEIGHTS (GRAM) SUMMARY
AFTER 13 WEEKS
FEMALES

		GROUP 1 0 PPM	GROUP 2 4500 PPM	GROUP 3 15000 PPM	GROUP 4 50000 PPM
BODY W.	MEAN	240.33	245.42	252.25	241.32
	ST.DEV.	12.46	16.71	8.80	14.34
	N	10	10	7	10
BRAIN	MEAN	1.91	1.94	1.95	1.89
	ST.DEV.	0.08	0.08	0.04	0.08
	N	10	10	7	10
HEART	MEAN	0.788	0.783	0.802	0.798
	ST.DEV.	0.056	0.067	0.044	0.058
	N	10	10	7	10
LIVER	MEAN	7.15	7.09	7.35	7.18
	ST.DEV.	0.85	0.61	0.72	0.53
	N	10	10	7	10
THYMUS	MEAN	0.28	0.29	0.33	0.27
	ST.DEV.	0.07	0.04	0.08	0.06
	N	10	10	7	10
KIDNEYS	MEAN	1.40	1.52	1.48	1.43
	ST.DEV.	0.12	0.14	0.13	0.13
	N	10	10	7	10
ADRENALS	MEAN	0.076	0.076	0.081	0.080
	ST.DEV.	0.011	0.012	0.011	0.014
	N	10	10	7	10
SPLEEN	MEAN	0.593	0.595	0.659	0.578
	ST.DEV.	0.118	0.102	0.111	0.084
	N	10	10	7	10
OVARIES	MEAN	0.110	0.109	0.119	0.106
	ST.DEV.	0.019	0.025	0.014	0.020
	N	10	10	7	10

*/**: Dunnett-test based on pooled variance sig. at 5% or 1% level.

ORGAN/BODY WEIGHT RATIOS SUMMARY
AFTER 13 WEEKS
FEMALES

		GROUP 1 0 PPM	GROUP 2 4500 PPM	GROUP 3 15000 PPM	GROUP 4 50000 PPM
BODY W.	MEAN	240.33	245.42	252.25	241.32
(GRAM)	ST.DEV.	12.46	16.71	8.80	14.34
	N	10	10	7	10
BRAIN	MEAN	0.80	0.79	0.77	0.78
(%)	ST.DEV.	0.03	0.06	0.02	0.04
	N	10	10	7	10
HEART	MEAN	0.328	0.320	0.318	0.331
(%)	ST.DEV.	0.019	0.026	0.014	0.026
	N	10	10	7	10
LIVER	MEAN	2.98	2.89	2.91	2.98
(%)	ST.DEV.	0.41	0.18	0.19	0.12
	N	10	10	7	10
THYMUS	MEAN	0.12	0.12	0.13	0.11
(%)	ST.DEV.	0.02	0.02	0.03	0.02
	N	10	10	7	10
KIDNEYS	MEAN	0.58	0.62	0.59	0.59
(%)	ST.DEV.	0.06	0.05	0.05	0.04
	N	10	10	7	10
ADRENALS	MEAN	0.032	0.031	0.032	0.033
(%)	ST.DEV.	0.005	0.005	0.005	0.006
	N	10	10	7	10
SPLEEN	MEAN	0.246	0.242	0.261	0.240
(%)	ST.DEV.	0.043	0.036	0.040	0.035
	N	10	10	7	10
OVARIES	MEAN	0.046	0.044	0.047	0.044
(%)	ST.DEV.	0.009	0.009	0.006	0.010
	N	10	10	7	10

*/**: Dunnett-test based on pooled variance sig. at 5% or 1% level.

HEMATOLOGY SUMMARY
MALES

	RBC T/l	HB mmol/l	HCT rel. 1	MCV fl	RDW rel. 1	MCH fmol	MCHC mmol/l
AFTER 13 WEEKS							
1 (0 PPM)	8.78	9.9	0.46	52.3	0.143	1.13	21.56
2 (4500 PPM)	8.84	9.8	0.46	52.0	0.135	1.11	21.40
3 (15000 PPM)	8.85	9.9	0.47	52.7	0.143	1.12	21.25
4 (50000 PPM)	8.96	9.9	0.47	52.3	0.134	1.11	21.20

	RETICULOCYTE COUNT					
	HDW mmol/l	RETIC. rel. 1	RETIC. G/l	L RETI rel. 1	M RETI rel. 1	H RETI rel. 1	WBC G/l
AFTER 13 WEEKS							
1 (0 PPM)	1.59	0.018	159	0.599	0.339	0.062	6.84
2 (4500 PPM)	1.57	0.018	162	0.610	0.334	0.056	6.70
3 (15000 PPM)	1.58	0.019	166	0.597	0.336	0.067	6.38
4 (50000 PPM)	1.52	0.021	186 **	0.577	0.353	0.070	7.85

	DIFF.WBC COUNT (REL)					
	NEUT. rel. 1	EOS. rel. 1	BASO. rel. 1	LYMPH. rel. 1	MONO. rel. 1	LUC rel. 1
AFTER 13 WEEKS						
1 (0 PPM)	0.255	0.014	0.003	0.700	0.020	0.009
2 (4500 PPM)	0.243	0.017	0.003	0.710	0.020	0.007
3 (15000 PPM)	0.236	0.016	0.003	0.716	0.018	0.011
4 (50000 PPM)	0.218	0.014	0.003	0.735	0.022	0.010

	DIFF.WBC COUNT (ABS)						
	NEUT. G/l	EOS. G/l	BASO. G/l	LYMPH. G/l	MONO. G/l	LUC G/l	PLATELETS G/l
AFTER 13 WEEKS							
1 (0 PPM)	1.75	0.09	0.02	4.78	0.14	0.06	855
2 (4500 PPM)	1.62	0.11	0.02	4.76	0.14	0.05	855
3 (15000 PPM)	1.54	0.10	0.02	4.54	0.12	0.07	908
4 (50000 PPM)	1.69	0.11	0.02	5.79	0.17	0.08	908

	COAGULATION	
	PT rel. 1	PTT sec
AFTER 13 WEEKS		
1 (0 PPM)	0.77	22.3
2 (4500 PPM)	0.77	23.4
3 (15000 PPM)	0.81	22.6
4 (50000 PPM)	0.80	23.2

*/**: Dunnett-test based on pooled variance sig. at 5% or 1% level. +: Steel-test sig. at 5% level.

HEMATOLOGY SUMMARY
FEMALES

	RBC T/l	HB mmol/l	HCT rel. 1	MCV fl	RDW rel. 1	MCH fmol	MCHC mmol/l
AFTER 13 WEEKS							
1 (0 PPM)	7.82	9.4	0.44	56.2	0.141	1.20	21.40
2 (4500 PPM)	7.95	9.5	0.45	55.9	0.130	1.20	21.43
3 (15000 PPM)	7.81	9.3	0.44	56.0	0.116	1.19	21.27
4 (50000 PPM)	7.87	9.4	0.44	56.1	0.128	1.19	21.23

	RETICULOCYTE COUNT						
	HDW mmol/l	RETIC. rel. 1	RETIC. G/l	L RETI rel. 1	M RETI rel. 1	H RETI rel. 1	WBC G/l
AFTER 13 WEEKS							
1 (0 PPM)	1.31	0.024	185	0.525	0.372	0.103	3.50
2 (4500 PPM)	1.35	0.022	173	0.544	0.367	0.089	4.02
3 (15000 PPM)	1.31	0.028	215	0.495	0.372	0.133	4.12
4 (50000 PPM)	1.30	0.023	184	0.516	0.366	0.118	4.73 *

	DIFF.WBC COUNT (REL)					
	NEUT. rel. 1	EOS. rel. 1	BASO. rel. 1	LYMPH. rel. 1	MONO. rel. 1	LUC rel. 1
AFTER 13 WEEKS						
1 (0 PPM)	0.204	0.018	0.002	0.749	0.019	0.008
2 (4500 PPM)	0.182	0.017	0.003	0.775	0.016	0.007
3 (15000 PPM)	0.194	0.021	0.003	0.758	0.018	0.007
4 (50000 PPM)	0.168	0.013	0.002	0.790	0.016	0.010

	DIFF.WBC COUNT (ABS)						
	NEUT. G/l	EOS. G/l	BASO. G/l	LYMPH. G/l	MONO. G/l	LUC G/l	PLATELETS G/l
AFTER 13 WEEKS							
1 (0 PPM)	0.72	0.06	0.01	2.62	0.07	0.03	951
2 (4500 PPM)	0.73	0.07	0.01	3.13	0.06	0.03	993
3 (15000 PPM)	0.80	0.08	0.01	3.13	0.08	0.03	980
4 (50000 PPM)	0.79	0.06	0.01	3.75 *	0.08	0.05 *	1007

	COAGULATION	
	PT rel. 1	PTT sec
AFTER 13 WEEKS		
1 (0 PPM)	0.79	29.3
2 (4500 PPM)	0.76	30.3
3 (15000 PPM)	0.78	29.3
4 (50000 PPM)	0.78	30.7

*/**: Dunnett-test based on pooled variance sig. at 5% or 1% level. +: Steel-test sig. at 5% level.

CLINICAL BIOCHEMISTRY SUMMARY
MALES

	GLUCOSE mmol/l	UREA mmol/l	CREAT µmol/l	BILI. T. µmol/l	CHOLEST.T. mmol/l	HDL-CHOL. mmol/l	LDL-CHOL. mmol/l
AFTER 13 WEEKS							
1 (0 PPM)	5.51	4.86	28.8	1.70	1.58	1.50	0.21
2 (4500 PPM)	5.67	5.57	31.0	1.42	1.62	1.52	0.22
3 (15000 PPM)	5.43	5.40	29.3	1.46	1.53	1.47	0.18
4 (50000 PPM)	5.03	4.72	27.8	1.63	1.50	1.43	0.19

	TRIGLY mmol/l	PHOS-LIP. mmol/l	ASAT U/l	ALAT U/l	ALP U/l	GGT U/l	CK U/l
AFTER 13 WEEKS							
1 (0 PPM)	0.36	1.34	84.1	34.9	65.3	0.0	155.5
2 (4500 PPM)	0.37	1.38	80.9	32.8	59.2	0.0	168.9
3 (15000 PPM)	0.35	1.31	83.6	32.3	66.4	0.0	175.2
4 (50000 PPM)	0.33	1.29	79.3	32.9	61.5	0.0	155.7

	SODIUM mmol/l	POTASSIUM mmol/l	CHLORIDE mmol/l	CALCIUM mmol/l	PHOSPHORUS mmol/l	PROTEIN T. g/l	ALBUMIN g/l
AFTER 13 WEEKS							
1 (0 PPM)	144.1	3.59	104.7	2.84	1.80	67.47	42.23
2 (4500 PPM)	144.5	3.71	105.6	2.85	1.74	67.24	42.25
3 (15000 PPM)	145.9 **	3.66	106.1 **	2.86	1.82	68.01	42.83
4 (50000 PPM)	146.2 **	3.56	105.5	2.90	1.96 *	66.31	42.13

	GLOBULIN g/l	A/G RATIO
AFTER 13 WEEKS		
1 (0 PPM)	25.25	1.68
2 (4500 PPM)	24.99	1.69
3 (15000 PPM)	25.17	1.71
4 (50000 PPM)	24.18	1.75

*/**: Dunnett-test based on pooled variance sig. at 5% or 1% level. +: Steel-test sig. at 5% level.

CLINICAL BIOCHEMISTRY SUMMARY
FEMALES

	GLUCOSE mmol/l	UREA mmol/l	CREAT µmol/l	BILI. T. µmol/l	CHOLEST.T. mmol/l	HDL-CHOL. mmol/l	LDL-CHOL. mmol/l
AFTER 13 WEEKS							
1 (0 PPM)	5.37	6.61	34.4	2.09	1.32	1.29	0.07
2 (4500 PPM)	5.99	6.22	32.8	2.09	1.39	1.37	0.07
3 (15000 PPM)	6.15	5.96	33.1	2.12	1.32	1.29	0.08
4 (50000 PPM)	5.59	6.13	32.2	2.06	1.28	1.25	0.09

	TRIGLY mmol/l	PHOS-LIP. mmol/l	ASAT U/l	ALAT U/l	ALP U/l	GGT U/l	CK U/l
AFTER 13 WEEKS							
1 (0 PPM)	0.26	1.41	78.5	27.6	23.7	0.0	143.6
2 (4500 PPM)	0.26	1.46	83.0	31.4	27.1	0.0	135.9
3 (15000 PPM)	0.23	1.33	78.6	25.0	24.4	0.0	181.3
4 (50000 PPM)	0.27	1.39	75.8	25.9	23.1	0.0	135.6

	SODIUM mmol/l	POTASSIUM mmol/l	CHLORIDE mmol/l	CALCIUM mmol/l	PHOSPHORUS mmol/l	PROTEIN T. g/l	ALBUMIN g/l
AFTER 13 WEEKS							
1 (0 PPM)	144.2	3.26	106.6	2.91	1.31	71.44	49.88
2 (4500 PPM)	144.5	3.27	106.7	2.86	1.43	70.48	48.68
3 (15000 PPM)	143.8	3.23	106.6	2.85	1.43	68.67	47.54
4 (50000 PPM)	144.9	3.35	107.5	2.90	1.54 *	69.49	47.77

	GLOBULIN g/l	A/G RATIO
AFTER 13 WEEKS		
1 (0 PPM)	21.56	2.32
2 (4500 PPM)	21.80	2.24
3 (15000 PPM)	21.13	2.25
4 (50000 PPM)	21.72	2.21

*/**: Dunnett-test based on pooled variance sig. at 5% or 1% level. +: Steel-test sig. at 5% level.

URINALYSIS SUMMARY
MALES

	VOLUME/18h ml	REL. DENS. rel. 1	pH	PROTEIN g/l	GLUCOSE mmol/l	KETONES mmol/l	UROBILI. µmol/l
AFTER 13 WEEKS							
1 (0 PPM)	13.3	1.029	6.5	0.18	0	0.3	0
2 (4500 PPM)	10.3	1.034	6.7	0.20	0	0.6	0
3 (15000 PPM)	15.2	1.028	6.8	0.18	0	0.4	0
4 (50000 PPM)	14.3	1.023	6.8	0.15	0	0.3	0

	BILIRUBIN µmol/l	ERY. per µl	LEU per µl
AFTER 13 WEEKS			
1 (0 PPM)	0	13	20
2 (4500 PPM)	0	12	28
3 (15000 PPM)	0	8	28
4 (50000 PPM)	0	6	15

*/**: Dunnett-test based on pooled variance sig. at 5% or 1% level. +: Steel-test sig. at 5% level.

URINALYSIS SUMMARY
FEMALES

	VOLUME/18h ml	REL. DENS. rel. 1	pH	PROTEIN g/l	GLUCOSE mmol/l	KETONES mmol/l	UROBILI. µmol/l
AFTER 13 WEEKS							
1 (0 PPM)	13.7	1.029	6.0	0.05	0	0.1	0
2 (4500 PPM)	15.1	1.028	6.1	0.08	0	0.2	0
3 (15000 PPM)	16.0	1.022	6.3	0.04	0	0.2	0
4 (50000 PPM)	11.2	1.027	5.9	0.05	0	0.1	0

	BILIRUBIN µmol/l	ERY. per µl	LEU per µl
AFTER 13 WEEKS			
1 (0 PPM)	0	2	0
2 (4500 PPM)	0	0	0
3 (15000 PPM)	0	0	0
4 (50000 PPM)	0	0	0

*/**: Dunnett-test based on pooled variance sig. at 5% or 1% level. +: Steel-test sig. at 5% level.

TEST ITEM	: α-glucosyl-hesperidin	PATH. NO.	: 11005 ROL
TEST SYSTEM	: Rat	DATE	: 15-NOV-05
STUDY TYPE	: 13-Week Oral Toxicity (Feeding)	SPONSOR	: Hayashibara Biochemical Laboratories, Inc

Histopathology

Histotechnique was performed at RCC Ltd, Itingen / Switzerland.

Slices of all organs and tissues indicated in bold face type in the list above collected at necropsy from all animals of the control and high dose group (group 1 and 4, respectively), and all gross lesions from all animals of all groups were processed, embedded in paraffin, cut at a nominal thickness of 2-4 micrometers, stained with hematoxylin & eosin (H&E) and examined by light microscope by the study pathologist.

Data Compilation

The animal data and necropsy findings were recorded on RCC-TOX Release 7.0, RCC Ltd, and were transferred electronically via transfer file into the PathData System V6.2.

The microscopic findings were recorded during histopathologic examination by the study pathologist and entered directly into the PathData System. The slides were evaluated during August 2005. Histological changes were described, whenever possible, according to distribution, severity and morphologic character. Severity scores were assigned as given under "Explanation of Codes and Symbols". In paired organs, findings occurred sometimes bilaterally and were of different degrees of severity. When this occurred, the higher degree was recorded and a comment was made which indicated the severity of the lesion in the contra lateral organ.

All microscopic findings are listed in the "TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS" (= AOFT, animal organ finding table), along with an explanation of the codes and symbols used. Computer-generated incidence tables derived from these data are also presented as well as the narrative of the both macroscopic and microscopic findings.

Data Cross Checking

The following sections were reviewed by Dr. Ph. Schaetti, (Toxicologic Pathologist, RCC Itingen, Switzerland): all organs from animals number 1, 31, 32, 45, 71 and 72.

RESULTS

Mortality

There were no premature deaths. All animals on study survived the scheduled study period.

Organ Weights

There were no differences indicating an effect of the test item.

Macroscopic Findings

At the end of the treatment period no test item-related gross lesions were observed. The macroscopic findings recorded were considered to be within the range of normal background lesions, which may be seen in species of this strain and age in this study type and were considered incidental, reflecting the usual individual variability.

TEST ITEM	: α-glucosyl-hesperidin	PATH. NO.	: 11005 ROL
TEST SYSTEM	: Rat	DATE	: 15-NOV-05
STUDY TYPE	: 13-Week Oral Toxicity (Feeding)	SPONSOR	: Hayashibara Biochemical Laboratories, Inc

Microscopic Findings

Minimal centrilobular hepatocelullar hypertrophy was recorded in two male animals from dose group 4.

A variety of other changes were found in this study. They commonly occur in laboratory species of this strain and age under the conditions of this study type. Neither their incidences nor their distribution or morphologic appearance gave any indication of a treatment-related association.

The detailed incidence data for all organs as well as tables of all changes including their severity appear in "SUMMARY INCIDENCE OF GRADINGS" and "NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS".

CONCLUSIONS

This study showed no premature deaths.

The fact that the three females (nos. 66, 68 and 70) had to be excluded from the study had no negative influence on the data presented.

Differences in organ weights, that could indicate an effect of the test item, were not recorded. Treatment-related gross lesions were not observed.

Microscopically minimal centrilobular hepatocelullar hypertrophy was recorded in two male animals from dose group 4. This finding was considered to be of adaptive character.

Under the conditions of this experiment, the test item produced no morphological evidence of toxicological properties in the organs and tissues examined.

MACROSCOPICAL FINDINGS SUMMARY
ALL NECROPSIES
MALES

	GROUP 1 0 PPM		GROUP 2 4500 PPM		GROUP 3 15000 PPM		GROUP 4 50000 PPM	
ANIMALS EXAMINED	10		10		10		10	
ANIMALS WITHOUT FINDINGS	10		9		9		10	
ANIMALS AFFECTED:								
SEMINAL VESICLES.....								
FOCUS/FOCI	0	0%	1	10%	0	0%	0	0%
SKIN.....								
NODULE(S)	0	0%	0	0%	1	10%	0	0%

/ ## : Fisher's Exact Test based on counts significant at 5% (#) or 1% (##) level

MACROSCOPICAL FINDINGS SUMMARY
ALL NECROPSIES
FEMALES

	GROUP 1 0 PPM		GROUP 2 4500 PPM		GROUP 3 15000 PPM		GROUP 4 50000 PPM	
ANIMALS EXAMINED	10		10		7		10	
ANIMALS WITHOUT FINDINGS	9		9		7		9	
ANIMALS AFFECTED:								
KIDNEYS.....								
PELVIC DILATION	1	10%	1	10%	0	0%	0	0%
BODY CAVITIES.....								
NODULE(S)	0	0%	0	0%	0	0%	1	10%

/ ## : Fisher's Exact Test based on counts significant at 5% (#) or 1% (##) level

TEST ARTICLE : Alpha-glucosyl-hesperidin *PATHOL. NO.*: 11005 ROL
TEST SYSTEM : RAT, 13-Week, Oral (Feeding) *DATE* : 15-NOV-05
SPONSOR : Hayashibara Biochemical Inc. *PathData@System V6.2b5*

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0

SEX :					MALE
DOSE GROUP:	01	02	03	04	
NO.ANIMALS:	10	10	10	10	
ADRENAL CORTICES :	10	-	-	10	
- Diffuse Fatty Change:	10	-	-	9	
- Diffuse Hypertrophy :	1	-	-	-	
BONE MARROW, FEMUR :	10	-	-	10	
- Fatty replacement :	9	-	-	9	
BRAIN STEM/MIDBRAIN :	10	-	-	10	
- Cyst :	1	-	-	-	
COLON :	10	-	-	10	
- Dilation :	-	-	-	1	
EPIDIDYMIDES :	10	-	-	10	
- Tubular Degeneration:	1	-	-	-	
EYES :	10	-	-	10	
- Hemorrhage :	4	-	-	2	
- Peribulbar inflam. :	1	-	-	2	
HEART :	10	-	-	10	
- Mononuclear Infiltr.:	2	-	-	2	
KIDNEYS :	10	-	-	10	
- Tubular Basophilia :	3	-	-	2	
- Hyaline Droplets :	6	-	-	10	
- Tubul.Hyperpl/Simple:	1	-	-	-	
- Papillary Mineraliz.:	2	-	-	-	
LIVER :	10	-	-	10	
- Fatty Change:Focal :	9	-	-	5	
- Mononuclear Infiltr.:	10	-	-	10	
- Centril.Hypertrophy :	-	-	-	2	

TEST ARTICLE : Alpha-glucosyl-hesperidin *PATHOL. NO.*: 11005 ROL
TEST SYSTEM : RAT, 13-Week, Oral (Feeding) *DATE* : 15-NOV-05
SPONSOR : Hayashibara Biochemical Inc. *PathData©System V6.2b5*

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0

SEX :				MALE
DOSE GROUP:	01	02	03	04
NO.ANIMALS:	10	10	10	10
LUNGS :	10	-	-	10
- Vascular Mineraliz. :	4	-	-	9
- Atelectasis :	2	-	-	2
- Alveol.Histiocytosis:	2	-	-	3
- Osseous Metaplasia :	-	-	-	1
MANDIB.LYMPH NODES :	10	-	-	10
- Lymphoid Hyperplasia:	9	-	-	9
MESENT. LYMPH NODE :	10	-	-	10
- Lymphoid Hyperplasia:	6	-	-	8
PEYERS PATCHES ILEUM :	10	-	-	10
- Lymphoid Hyperplasia:	10	-	-	10
PEYERS PATCHES JEJ. :	10	-	-	10
- Lymphoid Hyperplasia:	10	-	-	9
PROSTATE GLAND :	10	-	-	10
- Focal Hyperplasia :	-	-	-	1
RECTUM :	10	-	-	10
- Dilation :	1	-	-	2
SEMINAL VESICLES :	10	1	-	10
- Congestion :	-	1	-	-
SKIN/SUBCUTIS :	-	-	1	-
- Lipoma :	-	-	1	-
SPLEEN :	10	-	-	10
- Incr.Hemopoiesis :	10	-	-	10
STOMACH :	10	-	-	10
- Glandular Dilation :	-	-	-	1

TEST ARTICLE : Alpha-glucosyl-hesperidin *PATHOL. NO.*: 11005 ROL
TEST SYSTEM : RAT, 13-Week, Oral (Feeding) *DATE* : 15-NOV-05
SPONSOR : Hayashibara Biochemical Inc. *PathData©System V6.2b5*

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0

SEX :					MALE
DOSE GROUP:	01	02	03	04	
NO.ANIMALS:	10	10	10	10	
TESTES :	10	-	-	10	
- Sertoli C.Vacuolat. :	1	-	-	2	
THYMUS :	10	-	-	10	
- Atrophy :	10	-	-	9	
- Epithelial Cyst :	7	-	-	6	
THYROID GLAND :	10	-	-	10	
- Ultimobranchial Cyst:	1	-	-	-	
TRACHEA :	10	-	-	10	
- Glandular Ectasia :	2	-	-	1	

TEST ARTICLE : Alpha-glucosyl-hesperidin *PATHOL. NO.*: 11005 ROL
TEST SYSTEM : RAT, 13-Week, Oral (Feeding) *DATE* : 15-NOV-05
SPONSOR : Hayashibara Biochemical Inc. *PathData©System V6.2b5*

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0

SEX :					FEMALE
DOSE GROUP:	01	02	03	04	
NO.ANIMALS:	10	10	7	10	
ADRENAL CORTICES :	10	-	-	10	
- Diffuse Fatty Change:	10	-	-	10	
- Diffuse Hypertrophy :	2	-	-	2	
BODY CAVITIES :	10	-	-	10	
- Fat Necrosis :	-	-	-	1	
BONE MARROW, FEMUR :	10	-	-	10	
- Fatty replacement :	3	-	-	5	
BRAIN STEM/MIDBRAIN :	10	-	-	10	
- Cyst :	-	-	-	1	
COLON :	10	-	-	10	
- Dilation :	3	-	-	1	
EYES :	10	-	-	10	
- Hemorrhage :	1	-	-	1	
- Peribulbar inflam. :	-	-	-	1	
- Keratoconjunctivitis:	2	-	-	3	
KIDNEYS :	10	1	-	10	
- Tubular Basophilia :	2	-	-	-	
- Pelvic Dilation :	1	1	-	-	
- Tub.Lumin.Mineraliz.:	4	-	-	5	
- Tubular Vacuolation :	-	-	-	1	
LIVER :	10	-	-	10	
- Fatty Change:Focal :	3	-	-	2	
- Mononuclear Infiltr.:	8	-	-	8	
- Clear Focus :	2	-	-	-	
- Hematopoiesis :	1	-	-	-	
- Glycogen Increase :	5	-	-	4	

TEST ARTICLE : Alpha-glucosyl-hesperidin *PATHOL. NO.* : 11005 ROL
TEST SYSTEM : RAT, 13-Week, Oral (Feeding) *DATE* : 15-NOV-05
SPONSOR : Hayashibara Biochemical Inc. *PathData©System V6.2b5*

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0

SEX :					FEMALE
DOSE GROUP:	01	02	03	04	
NO.ANIMALS:	10	10	7	10	
LUNGS :	10	-	-	10	
- Vascular Mineraliz. :	3	-	-	3	
- Atelectasis :	-	-	-	1	
- Alveol.Histiocytosis:	3	-	-	2	
- Osseous Metaplasia :	1	-	-	-	
MANDIB.LYMPH NODES :	10	-	-	10	
- Lymphoid Hyperplasia:	8	-	-	9	
MESENT. LYMPH NODE :	10	-	-	10	
- Lymphoid Hyperplasia:	8	-	-	10	
OVARIES :	10	-	-	10	
- Stromal C.Hyperpl. :	1	-	-	2	
PEYERS PATCHES ILEUM :	9	-	-	10	
- Lymphoid Hyperplasia:	9	-	-	10	
PEYERS PATCHES JEJ. :	10	-	-	10	
- Lymphoid Hyperplasia:	10	-	-	6	
RECTUM :	10	-	-	10	
- Dilation :	2	-	-	2	
SCIATIC NERVE, LEFT :	10	-	-	10	
- Nerve Fiber Degener.:	2	-	-	1	
SPLEEN :	10	-	-	10	
- Incr.Hemopoiesis :	10	-	-	10	
- Hemosiderin Deposits:	-	-	-	1	
STOMACH :	10	-	-	10	
- Glandular Dilation :	-	-	-	3	
THYMUS :	10	-	-	10	
- Atrophy :	10	-	-	9	
- Epithelial Cyst :	9	-	-	9	

TEST ARTICLE : Alpha-glucosyl-hesperidin *PATHOL. NO.*: 11005 ROL
TEST SYSTEM : RAT, 13-Week, Oral (Feeding) *DATE* : 15-NOV-05
SPONSOR : Hayashibara Biochemical Inc. *PathData©System V6.2b5*

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0

SEX :					FEMALE
DOSE GROUP:	01	02	03	04	
NO.ANIMALS:	10	10	7	10	
THYROID GLAND :	10	-	-	10	
- Ultimobranchial Cyst:	-	-	-	1	
- Thymic remnant :	-	-	-	1	
TRACHEA :	10	-	-	10	
- Glandular Ectasia :	3	-	-	1	
- Mononuclear Infiltr.:	1	-	-	-	
VAGINA :	10	-	-	10	
- Cycle:Proestrus :	2	-	-	2	
- Cycle:Estrus :	5	-	-	2	
- Cycle:Metestrus :	3	-	-	6	

Expert Panel Report Regarding the Generally Recognized As Safe (GRAS) Status of The Intended Food Uses of Glucosyl Hesperidin

Introduction

Hayashibara International Inc. convened an independent panel of recognized experts (Expert Panel), qualified by their scientific training and relevant national and international experience to evaluate the safety of food and food ingredients, to determine the Generally Recognized As Safe (GRAS) status of Glucosyl Hesperidin for use as a food ingredient. The resumes of each Panelist are on file at the offices of Hayashibara International Inc. in Broomfield, Colorado. The qualifications of the Expert Panel satisfy the requirements set forth in the Federal Food, Drug, and Cosmetic Act's definition of generally recognized as safe (GRAS) substances (§ 201 (s)) and 21 CFR 170.30(a) "Eligibility for classification as generally recognized as safe (GRAS)."

Hayashibara International Inc. conducted a search of the published scientific literature on the safety/toxicity of Hesperidin and Glucosyl Hesperidin through September 2009 and the results were summarized and made available to the Expert Panel. In addition, Hayashibara International Inc. also made available internal Hayashibara reports (not published) and information on the manufacturing, product specifications, stability, intended uses and levels, and anticipated exposure intakes. The members of the Expert Panel independently and collectively critically evaluated information provided by Hayashibara International Inc. and other materials deemed appropriate or necessary. Following this independent and critical evaluation, the Expert Panel conferred by telephone on September 24th, 2009, with representatives of Hayashibara International Inc. The information critically evaluated by the Expert Panel was compiled into a GRAS Report, which supports the eligibility of Glucosyl Hesperidin as a GRAS ingredient in accordance with 21 CFR 170.36. The information provided for the Expert Panel's review was presented in the form stipulated in 21 CFR 170.35. The Expert Panel unanimously concluded on

September 24th, 2009 that the intended uses of Glucosyl Hesperidin as a food ingredient when produced in accordance with the *"Specifications and Standards for Food, Food Additives, etc. under the Food Sanitation Law in Japan"*, used in accordance with current Good Manufacturing Practice (cGMP), and meeting the specifications described herein, are GRAS based on scientific procedures.

Notice of GRAS exemption claim

Glucosyl Hesperidin is exempt from pre-market approval requirements of the Federal Food, Drug and Cosmetic Act because it has been determined to be generally recognized as safe (GRAS) under conditions of intended use by experts qualified by scientific training and experience.

Summary of the Basis of the GRAS Determination

Chemistry and Manufacturing

- Glucosyl Hesperidin (GH) is a derivative of hesperidin on which a single glucose molecule is attached to the rhamnosylglucoside (rutinose) moiety of hesperidin.

- GH is produced using a cyclodextrin glucanotransferase (EC 2.4.1.19), which is isolated from a strain of *Bacillus stearothermophilus.* This enzyme transfers a glycosyl group (1 or more glucose units) to the rhamnosylgucoside moiety (C4 position of glucose) of hesperidin. Excess glucose units are cleaved off using glucoamylase (EC 3.2.1.3) until a single terminal glucose unit is left. GH is manufactured by Hayashibara Company, Ltd., Okayama, Japan, according to the "*Specifications and Standards for Food, Food Additives, etc. under the Food Sanitation Law in Japan.*"

- The resulting end product, GH, mainly consists of not less than 70% monoglucosyl hesperidin (MGH; MW = 772.70), of which a single glucose

molecule is attached to the rhamnosylglucoside moiety of hesperidin. GH also includes unreacted hesperidin, small amounts of glycosyl hesperidin (having 2 or more glucose units attached to the rhamnosylglucoside moiety of hesperidin), and free saccharides.

- GH comes in the form of a powder with a light yellow to yellowish brown color. The principal difference from hesperidin, besides the additional glucose unit, is its water solubility, which is more than 10,000 times greater than natural hesperidin.

- Final product specifications for GH are shown below (Table 1).

Table 1 Final Product Specifications for Glucosyl Hesperidin

Variables	Specifications
Appearance	Light yellow to yellowish brown powder with slight characteristic odor
Identification (1)	The solution turns into brown.
(2)	Identify the maximum absorption at 280 – 286 nm.
Lead	Not more than 0.1 μg/g
Loss on drying	Not more than 6.0%
Residue on ignition	Not more than 2.0%
pH	5.0 – 7.0
Viable count	Not more than 300 CFU/g
Coliform organisms	Negative/0.1 g
Purity of MGH	Not less than 70.0% (on the dried base)
Total hesperidin	Not less than 70.0% (on the dried base)

- An analysis of five lots of GH demonstrates that the specifications can be consistently met.

- Heavy metal and microbial analyses of GH demonstrate that it does not contain toxicants of concern. Lead, mercury, cadmium and arsenic levels from 5 lots of GH were less than 0.1 ppm (the limit of detection). All samples of GH from 5 lots were negative for *P. aeruginosa*, *E. coli*, *S. aureus*, and *Salmonella* (sp.).

Functionality, Uses, and Exposure

- Glucosyl Hesperidin (GH) is a derivative of hesperidin, which is a major flavonone. As with other bioflavanoids, GH can function as an antioxidant in foods to stabilize colors and flavors. With its high solubility, GH can be included in many food applications where hesperidin cannot be used because of its low solubility. Other functional uses include stabilization of sedimentation of natural hesperidin, which commonly precipitates during normal processing of orange juice, or during storage of orange juice containing liquids. It can also be used to add color and flavor to products. Because it is metabolized like hesperidin, it can also be added to foods as a dietary antioxidant.

- GH has been used in commercial food products in Japan since 1997. Total consumption has been about 70 metric tons, and no consumer complaints are known to Hayashibara. Its history of 12 years of use in products consumed by humans supports the conclusion that it is safe. GH is listed in the *List of Existing Food Additives* (1996), issued by the Japanese Ministry of Health, Labor and Welfare (MHLW), and in the 8th edition of *Japan's Specifications and Standards of Food Additives* (2007). It is also approved in Korea as a food additive.

- GH may be used in various food categories which are defined by 21 CFR §170.3 (n) for a variety of Technical Effects, which are codified at 21 CFR §170.3 (o) (1-32). Expected technical effect categories include:

  (3) Antioxidants
  (4) Colors and coloring adjuncts

(11) Flavor enhancers
(12) Flavoring agents and adjuvants
(20) Nutrient supplements
(24) Processing aids

- The estimated daily mean exposure derived from calculations based on USDA's *Continuing Survey of Food Intakes by Individuals (1996)* is 0.695 grams per day (approximately 12 mg/kg bw/day for a 60 kg person). The 90th percentile is then estimated to be 1.390 grams per day (approximately 23 mg/kg bw/day for a 60 kg person)

Metabolism of Glucosyl Hesperidin in Animals and Humans

Four studies in rats and one study in humans were conducted using Glucosyl Hesperidin (GH), which has as its major constituent monoglucosyl hesperidin (MGH). Ingestion of GH showed that it was hydrolzyed first to hesperidin in the upper digestive tract. The hesperidin was then further hydrolyzed to hesperetin, primarily in the lower intestine. Hesperetin is then absorbed into the blood, and either at the lumen wall or in the liver the hesperetin is conjugated with glucuronic acid. There are also other conjugation molecules, but it appears glucuronic acid is the principal one. Except for the initial hydrolysis of GH to hesperidin in the upper intestinal tract, the absorption into the body of hesperetin derived from GH is essentially the same as that of natural hesperidin.

- Hesperidin studies on rats and humans have shown that hesperidin is hydrolyzed to its aglycone form, hesperetin, in the lower intestinal tract. Some of the hesperetin enters the enterocytes and is conjugated with glucuronic acid within these cells. It is also extracted into the hepatocyte from the portal circulatory system where it is also undergoes glucuronidation. The plasma T_{max} of hesperetin-glucuronide in the blood of humans given natural hesperidin has been

reported as approximately 5 to 7 hours (Manach, et al., 2003; Erlund, et al., 2001, Nielsen et al., 2006).

- To investigate the metabolism of GH in rats, GH was dissolved in distilled water and given by gastric intubation at a dose of 1 mmol/kg-bw (Yamada, et al., 2006a). After GH administration, hesperetin-glucuronide was found in the serum with the T_{max} being 6 hours. The serum T_{max} for hesperetin-glucuronide following gastric intubation of hesperidin was 9 to 12 hours. The serum $AUC_{0\text{-}25\ hrs}$ of hesperetin-glucuronide in rats given GH was 3.7 times greater than for rats administered hesperidin. This is likely caused by the large difference in solubility of the two substances.

  Enzymatic activity of either a small intestine homogenate (SIH), or of cecal contents (CC) showed similar α-glucosidase hydrolytic activity; however, β-glucosidase hydrolytic activity was much higher in the CC sample than the SIH. *In vitro* incubation of GH with SIH and CC demonstrated that GH hydrolysis to hesperidin occurred with the SIH, but no hesperetin was formed. However, incubation with CC resulted in the hydrolysis of GH to hesperidin and then hesperidin to hesperetin. This suggests that the additional glucose in the GH is hydrolyzed from the GH molecule to form hesperidin plus free glucose in the upper intestine, but for the rutinose moiety to be cleaved, the molecule must interact with the β-glucosidase located in the lower intestine (Yamada, et al., 2006a).

- GH and hesperidin, at doses of 1 mmol/kg-bw in 2 mL of water were administered to rats by stomach intubation. The stomach, jejunum, ileum, caecum, liver, and kidneys were collected from subgroups at time 0, 6 and 24 hours after administration. Digestive tissues were collected and assayed with their contents. Fecal samples were collected for 24 hours after administration. GH, hesperidin and hesperetin were assayed in each sample collected. In the tissue/content homogenates of the stomach, jejunum, ileum, cecum, liver and

kidney, hesperidin and hesperetin at 6 hours were greater for the animals administrated GH than those given hesperidin. Except in the stomach, there was little of either hesperidin or hesperetin in any of the samples at 24 hours after administration. The feces contained μmol amounts of hesperidin (about 1.6 μmol/g) and hesperetin (about 0.6 μmol/g). The data suggest that administration of GH in water results in relatively more hesperidin and hesperetin being found in all the tissue samples at 6 hours, than when hesperidin is administrated. The data also suggest that hesperidin and hesperetin concentrations were greatly reduced in all tissues, except the stomach by 24 hours. The reason for the increase in hesperetin in the stomach tissue at 24 hours after administration is not known. Further these data show that under the conditions of this study not all the hesperetin is absorbed, but rather was excreted in the feces (Yamada, et al., 2006b).

- GH and hesperidin (0.5 mmol/kg-bw) were suspended in a 2-mL aqueous solution of 0.2% CMC-Na (CMC), and administered by stomach intubation. The purpose of using CMC was an attempt to equalize the solubility of the two substances. Serum, urine, feces, liver and kidney samples were collected from subgroups at several times after administration. The serum T_{max} of hesperetin-glucuronide for both administration groups was reached at 9 hours, and the serum $AUC_{0\text{-}27\ hrs}$ of hesperetin-glucuronide was essentially the same. Approximately 90% of the hesperetin in the serum of both administration groups was glucuronidated. Essentially no hesperidin was detected in the liver, kidneys, urine or feces at any time. Hesperetin was detected at the 9-hour sampling, and to a lesser extent at 27 hours in liver and kidney samples of both administration groups. Hesperetin was also detected at similar concentrations in the urine and feces of rats given either hesperidin or GH. The data demonstrate that if GH and hesperidin are administered in a CMC matrix, the amount of intra-lumenal hesperetin created is absorbed in equal amounts, and the subsequent distribution, metabolism and excretion are the same (Mitsuzumi, et al., 2006).

- A second study was performed in an identical manner to the previous study (Mitsuzumi, et al., 2008). Stomach, jejunum, ileum and cecum, with their contents, were collected from subgroups of the treated rats at various times. The pattern of concentrations of hesperidin and hesperetin in the jejunum, ileum and cecum samples was similar for the GH and hesperidin administration groups. In rats administrated GH, most of GH was hydrolyzed into hesperidin in the jejunum by 3 hours. Although only a minor amount of hesperidin was converted to hesperetin in the ileum, there was a significant ($p < 0.05$) difference in the amount of conjugated versus non-conjugated hesperetin at the 6 and 9 hour sampling times for both administration groups. This suggests that a portion of the hesperetin in the ileum is being absorbed. The hesperidin in both the GH and hesperidin groups is subsequently converted into hesperetin in the cecum from 3 to 24 hours. At the 12-hour sample, essentially no GH or hesperidin was detected in the cecum and no conjugated hesperetin was detected. At this time, it is likely that the GH and hesperidin are excreted in the feces and not converted to hesperetin because the concentration of hesperetin does not increase in a proportional manner. These data suggest that if hesperidin, hydrolyzed from GH, and natural hesperidin are absorbed in the same amount, the relative concentrations of hesperidin and hesperetin in the digestive tract are similar. The results further indicate that the absorption of hesperetin, derived from GH, is essentially identical to that from natural hesperidin.

- In a human study, 10 healthy adults (5 female/5 male; 37.1 $\pm$ 8.8 years) were given one dose of 500 mg of GH in 50 mL of water (Yamashita, et al., 2008). The plasma samples had no free hesperidin or hesperetin, and all hesperetin was glucuronidated. No hesperetin-glucuronide was detected in the plasma through three hours after administration. The peak concentration of plasma hesperetin-glucuronide was between 6 and 8 hours after administration. This generally compares to other studies in which hesperidin was given to humans. In a second study, the same subjects were given the same dose (500 mg) of GH daily for 14 consecutive days. Plasma concentrations of hesperetin (glucuronidated)

obtained at 1, 7 and 14 days after the start of ingestion were similar (not significantly different). Seven days after stopping ingestion, no hesperetin-glucuronide was detected in plasma. These data demonstrate that ingestion of GH results in a similar pattern of hesperetin-glucuronide plasma concentration as when natural hesperidin is consumed. In addition, daily consumption of GH does not result in accumulation of hesperetin-glucuronide in the plasma. Also, hesperetin-glucuronide cannot be detected in the plasma 7 days after cessation of ingestion of GH.

Safety Studies

Safety studies in animals, a number of safety studies in humans, and a history of safe human consumption in Japan (> 10 years) support the safety of Glucosyl Hesperidin.

Animal Safety Studies

- An acute study in mice (HanIbIm:WIST [SPF]; 5/sex) demonstrated that a single dose of Glucosyl Hesepridin (GH) orally administered at doses up to 2,000 mg/kg-bw (the OECD limit dose) did not cause adverse clinical signs or deaths. (Arcelin, 1997).

- A 4-week subacute oral toxicity study of GH in rats (HanBrl:WIST [SPF]; 5/sex/treatment group) showed a no observable adverse effect level (NOAEL) at 15,000 ppm, the highest concentration tested, which is equivalent to 1,205 and 1,279 mg/kg-bw/day for male and female rats, respectively (Pipp, Krinke, 2005).

- A thirteen-week subchronic oral toxicity study of GH was conducted in rats (HanRcc:WIST [SPF]; 10/sex/treatment group) and the NOAEL was 50,000 ppm, the highest concentration tested. This is equivalent to 3,083 mg/kg-bw/day for males and 3,427 mg/kg-bw/day for females. No treatment-related dose-

dependent and clinically adverse effects were reported for any of the variables evaluated (Sommer, et al., 2005).

- The reproductive and developmental effects of GH were examined in 80 pregnant rats (CRL:CD(SD) [SPF]) orally administrated doses of 0, 100, 300 or 1,000 mg/kg-bw/day from days 6 to 17 of gestation (Itoh, 2007). No dose-dependent developmental toxicity was apparent in any of the fetuses, and the no observable adverse effect level (NOAEL) for maternal and developmental toxicity was 1,000 mg/kg/day, the highest dose tested.

- GH was not mutagenic in either *Salmonella typhimurium* or *Escherichia coli* strains in the absence or presence of rat S9 microsomal fraction (Wollny, 1997). The sample of GH used in this study had been enzymatically modified but contained MGH, and a metabolite (hesperetin-7-glucoside), but no hesperidin.

- A chromosomal aberration study of GH using Chinese hamster cells found no genotoxicity up to 5,000 µg/mL (Zheng, 2006a). In a micronucleus test, no significant increase in the frequency of micronucleated PCE was observed in mice after administration by gastric intubation of GH at doses of 0, 500, 1000 or 2,000 mg/kg-bw (Zheng, 2006b).

- Four studies were designed to assess the short and long-term effect of GH consumption in specific strains (SHR:Izm and WKY:Izm) of male rats (Ohtsuki, et al., 2002, 2003; Yamamoto, et al., 2008a, 2008b). Test groups were administered GH diets at concentrations designed to deliver doses of 10 to 50 mg/kg-bw of GH from 12 hours to 25 weeks. While not the primary purpose of these studies, there were no treatment-related dose-dependent adverse effects on any of the variables evaluated. Most of evaluations were of a limited nature and not all variables were examined in all four studies. The studies included data on body weight, food intake, blood pressure, heart rate, food consumption, body weight gain, clinical chemistry, hematology, histology, urine volume and

chemistry, and organs weights. No consistent, statistically significant, dose-dependent adverse effects were reported in any study.

Human Safety Studies

It has been demonstrated in several studies that the metabolism of hesperidin and Glucosyl Hesperidin (GH), except for the initial hydrolysis of the added glucosyl unit, are identical in all species studied. The initial removal of the glucose occurs early in the upper intestinal tract and does not interfere with the absorption in the lower intestine. The difference is principally the kinetics of uptake with the disparity caused by the higher water solubility of GH compared to hesperidin. Both substances are absorbed as hesperetin. When the solubility was equalized using CMC (carboxymethyl cellulose), the two substances were absorbed and metabolized in an identical manner (Yamada, et al., 2006a; Yamada, et al., 2006b; Mitsuzumi, et al., 2006; Mitsuzumi, et al., 2008). These findings strongly suggest that the consumption of GH would result in no greater concern for humans than the consumption of natural hesperidin.

Hesperidin has been consumed as a natural part of foods, principally citrus fruits and foods containing citrus fruits, since prehistoric times. Many safety studies have been preformed using hesperidin and/or hesperidin containing complexes (Fisher, 1982, Pirzzorno Jr, Murray, 1999, Grag, et al., 2001). The Life Sciences Research Offices evaluated the safety of hesperidin for the US FDA (Contract number FDA 223-78-2100) and determined that, "There is no evidence in the available information on hesperidin (purified or hesperidin complex) or naringin that demonstrates, or suggests reasonable grounds to suspect hazard to the public when they are used at levels that are now current or that might reasonably be expected in the future" (Fisher, 1982).

The safety of GH has also been tested in several placebo- and non-placebo-controlled human studies. The results of these studies do not raise any concerns about the safety of this substance. The studies are summarized below.

- Miwa and co-workers performed two studies in which a total of 65 adult male subjects (45.7 ± 10.5 and 44.1 ± 10.7 years) were given doses of up to 500 mg of GH in tablet form daily for up to 24 weeks (Miwa, et al., 2004; Miwa, et al., 2005). While these two studies were not designed as safety studies, the available information on limited serum chemistry tests suggests no safety related concerns. Additionally, the authors did not mention the occurrence of any adverse effects.

- A study was performed in which three groups of healthy adult subjects (n = 51, 19, and 13; male/female, 54/29; 43.1 ± 8.7 years) were given a tea beverage containing 340, 340, or 1,020 mg of GH per day for 12, 12, or 4 weeks, respectively (Yuasa, et al., 2005). Detailed hematologic and biochemical values were obtained before, during and at 4 weeks after the administration period. The author's concluded that, "No adverse effects derived from the intakes of GH-beverage were noticeable throughout each study."

- A randomized, double-blind placebo-controlled study was performed using a total of 167 male and female volunteers (male/female, 82/85; 49.4 ± 9.0 years) (Kozuma, et al., 2007). Approximately 1/2 of the subjects received 35 mg of MGH (in this study termed mGHES) consumed in 15 mL low sodium soy sauce daily for 12 weeks. The control group consumed only the 15 mL low sodium soy sauce. None of the biochemical variables tested showed any signs of adverse effects. Evaluation of adverse events revealed that the placebo control group actually had a consistently greater number of adverse events than the GH group. Nevertheless, all adverse events reported were not serious and resolved within a few days. The authors concluded that, "Results of adverse effects during mGHES [MGH] treatment were relatively low and were similar in both group."

- A group of 119 male and female subjects categorized as "non-lean" were enrolled in a randomized, double-blind, placebo-controlled study (Hanawa, et al., 2008). Four (4) subjects withdrew for personal reasons before treatment was initiated, and therefore 115 subjects were included in the safety analysis. The mean age was 45.0 ± 7.8 years and the number of male to female subjects was 81 to 34, respectively. One group was given tablets containing 500 mg of GH daily for 12 weeks. The control group received placebo tablets for the same length of time. Three separate adverse events were observed in the GH group in one subject each, namely changes of liver function values, systemic skin rash, and hypogeusia. The authors suggested that these signs and symptoms should be monitored in future studies, although similar adverse events have not been observed in any other studies. The authors noted that, "No other significant adverse events were found on physical, blood or urine examinations or interview."

- Nakagawa and coworkers performed a two part study (Nakagawa, et al., 2008). In the first study, 85 subjects (40 males and 45 females; 48.5 ± 11.0 years) were randomized into 2 groups. One group consumed a green tea drink containing 500 mg of GH daily for 12 weeks. The control group had a placebo added. In the second study, a total of 28 male (16) and female (12) subjects (38 ± 11 years) consumed 1.02 g of GH daily in the green tea drink for 4 weeks. The authors stated that, "Throughout these trials, no adverse effects was [sic] found in physiological, hematological, biochemical parameters and in medical interviews. In conclusion, this study suggested that GTP [green tea powder] added G-HSP [GH] ..... showed no adverse effects in long-term and excessive ingestion."

- Hayashibara Company, Ltd. developed the GH production method in 1989 and has been manufacturing, packaging, storing and transporting GH and the materials used to produce this substance on a commercial basis since 2005. No

adverse event related to regular and direct contact to the finished product or related substances was reported by any employee (Kibata, 2007).

Conclusion

We, the Expert Panel, have independently and collectively critically evaluated the information and data on Glucosyl Hesperidin summarized in the GRAS Report, and other information deemed necessary, and conclude, that the intended uses of Glucosyl Hesperidin as a food ingredient, produced in accordance with the "Specifications and Standards for Food, Food Additives, etc. under the Food Sanitation Law in Japan", meeting the specifications described herein, and used in accordance with cGMP, are Generally Recognized As Safe (GRAS) based on scientific procedures.

It is our opinion that other qualified experts would concur with this conclusion.

Professor Joseph F. Borzelleca, Ph.D., F.A.T.S., Chairman — Date
Virginia Commonwealth University School of Medicine

[redacted] — 20 Nov 2009
Professor I. Glenn. Sipes, Ph.D., F.A.T.S. — Date
University of Arizona School of Medicine

[redacted] — 20 Nov 2009
Professor John A. Thomas,Ph.D., F.A.T.S., D.A.C.T. — Date
Indiana University School of Medicine

One hundred and five pages have been removed in accordance with copyright laws. The removed reference is:

Masaaki Hanawa, Reduction of body weight and body fat during long-term consumption of foods containing glucosyl hesperidin, Journal of Nutritional Food, Vol. 11, No. 3, 2008, Pages 1-105.

HAYASHIBARA
NAGASE Group

Jun 22, 2020

Statement

Dear FDA GRAS Notice staff:

This document is in further response to one of the questions provided by the FDA regarding GRAS Notice GRN 000901, in a letter dated May 4, 2020. The question was in the section **Chemistry review** item 5., regarding analytical methods established in Japanese reference standards (Japan's Specifications and Standards for Food Additives, Japan Industrial Standard, and Japanese Pharmacopoeia).
Hayashibara Co., Ltd., the Notifier of GRAS Notice 000901, confirms that all the analytical methods used from Japanese standard references are validated for use in the determination of the respective analytes.

Sincerely,

Keisuke Namekawa
Unit Manager
Regulatory Affairs Unit
Hayashibara Co., Ltd.

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https://www.hayashibara.co.jp/en/

From:	Richards Alan
To:	Kolanos, Renata
Subject:	GRN 00901 - Response to FDA question
Date:	Monday, July 27, 2020 7:33:09 PM

Dear Dr. Kolanos:

This email is in response to the phone conference between the FDA and Hayashibara Co., Ltd., regarding GRN 00901 Glucosyl Hesperidin (GH). The called was on July 22, 2020 at 10:00 am EDT.

The item for discussion was a follow-up to one of a series of questions the FDA reviewers had about GRN 00901 in a letter dated May 4, 2020. In Hayashibara's response the Notifier could only provide the product names for the two ion-exchange resins used for mixed-bed processing of the GH. Both resins meet the "Ion Exchange Resins" monograph in Japan's Specifications and Standards for Food Additives of the Food Sanitation Law. These were Amberlyst™ 16WET and Amberlite™ IRA96SB, both of which are now owned by Dow.

The Japanese distributor of these products has provided Hayashibara with additional information from the manufacture.

Review of Amberlyst™ 16WET describes it as a copolymer of styrene-divinylbenzene with a sulfonic acid functional group. This is consistent with the approved ion-exchange resin as listed in 21CFR 173.25 (a)(1). However, the Japanese distributor informed Hayashibara that the resin is not guaranteed to meet the FDA regulation because the specified extraction tests have not been performed.

Amberlite™ IRA96SB, which is a copolymer of styrene-divinylbenzene with a functional tertiary amine, is not consistent with 21CFR 173.25. The reason given by the manufacturer was that this ion-exchange resin was manufactured before establishment of the FDA regulation and has not been added to the list.

As mentioned in GRN 00901, Part 6.2, GH has been commercially sold in Japan for approximately 22 years, with no reports, to the Notifier's knowledge, of any untoward effects to manufacturing personnel, finished products personnel, or customers of the end products. Further, the safety of the products was examined in a 13-week sub-chronic toxicity study and shown to be safe, even at the highest dose (mean NOAL 3,256 mg/kg/day).

Hayashibara Co., Ltd., awaits the Agencies comments on this matter.

Sincerely,

Alan B. Richards, PhD
Vanguard Regulatory Services, Inc.
1311 Iris Circle
Broomfield, CO 80020
Tel: +1-303-464-8636
Mob: +1-720-989-4590
vrsi@comcast.net

From:	Richards Alan
To:	Kolanos, Renata
Subject:	Glucosyl hesperidin - GRN No. 000901 - Response to FDA request
Date:	Monday, September 07, 2020 4:03:21 PM
Attachments:	FDA Responce Statement_200831.pdf New Resins.pdf

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Dear Dr. Kolanos:

This email and attachments are in response to your email of August 7, 2020 concerning the use of alternative ion exchange resins for the production of glucosyl hesperidin (GRN No. 000901). In your email you provided the Notifier, Hayashibara Co., Ltd., with the type of statements needed to confirm that the alternative ion exchange resins selected were satisfactory for use in the manufacture of the ingredient.

Hayashibara Co., Ltd. has performed the necessary analyses to make the confirmatory statements requested by the Agency. Please find them attached.

Please contact me if you have any questions or comments.

Sincerely, Alan

Alan B. Richards, PhD
President
Vanguard Regulatory Services, Inc.
1-303-464-8636 (o)
1-720-989-4590 (m)
vrsi@comcast.net

Advisor - Technical & Regulatory
Hayashibara Co., Ltd.
675-1 Fujisaki, Naka-ku
Okayama 702-8006, Japan

August 31, 2020

Dear FDA GRAS Notice staff:

Statement

We, Hayashibara Co., Ltd., confirm the following:

1. The alternative ion exchange resins used in the manufacture of glucosyl hesperidin are authorized for their intended use in the U.S. as attached.

2. Glucosyl hesperidin manufactured using the alternative resins meets the specifications provided in the GRAS Notice.

3. The change in the manufacture of glucosyl hesperidin does not impact the identity or composition of the ingredient, the relevance of studies in Hayashibara's safety narrative, and Hayashibara's GRAS conclusion for this ingredient under its intended conditions of use.

Sincerely,

Yoshikatsu Miwa
Unit Manager
Quality Assurance Unit

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http://www.hayashibara.co.jp/en

Document No. IER01-76-055
July 29th, 2020

Organo Corporation
Functional Materials Department

To Hayashibara Co., Ltd.

·AMBERLITE™ FPC20 Na

This product complies with the U.S. Food and Drug Administration's Food Additive Regulation 21 CFR 173.25(a)(1). Use of this product is subject to good manufacturing practices and any limitations which are part of the regulations. The regulations should be consulted for complete details.

·AMBERLITE™ FPA95

This product complies with the U.S. Food and Drug Administration's Food Additive Regulation 21 CFR 173.25(a)(5). Use of this product is subject to good manufacturing practices and any limitations which are part of the regulations. The regulations should be consulted for complete details.

The above information relates specifically to the product reviewed. We recommend customers make their own determination of resin suitability for their particular intended use(s). We believe this information is reliable as of the date of this letter.

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