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EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA)

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SCIENTIFIC OPINION

Safety of glucosyl hesperidin as a Novel food pursuant to Regulation (EU) 2015/2283

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Abstract

Following a request from the European Commission, the EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA) was asked to deliver an opinion on glucosyl hesperidin (GH) as a novel food (NF) pursuant to Regulation (EU) 2015/2283. The NF, which is produced from hesperidin and dextrin by enzymatic reactions, is a powder consisting mainly of monoglucosyl hesperidin (MGH) and unreacted hesperidin (flavonoid), which account in total for up to 92.8% (on dry basis) of the product. The applicant proposed to use the NF in specific drinks and food supplements leading to a maximum intake of up to 364 mg per day for adults. The target population is the general population, except for food supplements for which the proposed target population is children from 1 year onwards and adults. Taking into consideration the composition of the NF and the proposed uses, the consumption of the NF is not nutritionally disadvantageous. There are no concerns regarding genotoxicity of the NF. Based on a 90-day oral toxicity study conducted with the NF, the Panel considers the NOAEL at the mid-dose group, i.e. ~1000 mg/kg body weight (bw) per day. By applying an uncertainty factor of 200, the resulting intake providing sufficient margin of exposure for humans would be 5 mg/kg bw per day. The available human intervention studies did not report clinically relevant changes in haematological or clinical chemistry parameters following the administration of GH/MGH at supplemental doses of up to 3 g/day for 12 weeks. Overall, the Panel considers that the margin of exposure (~200) between the intake of the NF at the proposed uses and use levels and the NOAEL from the 90-day study is sufficient. The Panel concludes that the NF, glucosyl hesperidin, is safe for the target population at the proposed uses and use levels.

KEYWORDS

enzymatic reaction, food supplements, glucosyl/monoglucosyl hesperidin, hesperidin, novel foods

CONTENTS

1 | INTRODUCTION

1.1 | Background and Terms of Reference as provided by the requestor

On 26 March 2021, the company Hayashibara Co., Ltd.[1] submitted a request to the Commission in accordance with Article 10 of Regulation (EU) 2015/2283 to place on the EU market glucosyl hesperidin.

Glucosyl hesperidin is intended to be used for addition to several hot beverages, non-alcoholic beverages, confectionery and as food supplements as defined in Directive 2002/46/EC.

In accordance with Article 10(3) of Regulation (EU) 2015/2283, the European Commission asks the European Food Safety Authority to provide a scientific opinion on glucosyl hesperidin. In addition, the European Food Safety Authority is requested to include in its scientific opinion a statement as to if, and if so to what extent, the proprietary data for which the applicant is requesting data protection was used in elaborating the opinion in line with the requirements of Article 26(2)(c) of Regulation (EU) 2015/2283.

2 | DATA AND METHODOLOGIES

2.1 | Data

The safety assessment of this NF is based on data supplied in the application and information submitted by the applicant following EFSA's requests for supplementary information.

Administrative and scientific requirements for NF applications referred to in Article 10 of Regulation (EU) 2015/2283 are listed in Commission Implementing Regulation (EU) 2017/2469.[2]

A common and structured format on the presentation of NF applications is described in the EFSA guidance on the preparation and presentation of an NF application (EFSA NDA Panel, 2016). As indicated in this guidance, it is the duty of the applicant to provide all of the available (proprietary, confidential and published) scientific data (including both data in favour and not in favour) that are pertinent to the safety of the NF.

This NF application includes a request for protection of proprietary data in accordance with Article 26 of Regulation (EU)2015/2283. The data requested by the applicant to be protected comprise: (i) production process; (ii) composition and stability of the NF; and (iii) toxicological information.

2.2 | Methodologies

The assessment follows the methodology set out in the EFSA guidance on NF applications (EFSA NDA Panel, 2016) and the principles described in the relevant existing guidance documents from the EFSA Scientific Committee. The legal provisions for the assessment are laid down in Article 11 of Regulation (EU) 2015/2283 and in Article 7 of Commission Implementing Regulation (EU) 2017/2469.

This assessment concerns only the risks that might be associated with consumption of the NF under the proposed conditions of use and is not an assessment of the efficacy of the NF with regard to any claimed benefit.

3 | ASSESSMENT

3.1 | Introduction

The NF falls under Article 3 of Regulation (EU) 2015/2283, i.e. food with a new or intentionally modified molecular structure and food consisting of, isolated from or produced from plants and their parts.

The NF, which is the subject of the application, is a pale yellow to yellow-brown powder consisting of monoglucosyl hesperidin (75%–85% on dry matter basis) and produced from hesperidin and dextrin by enzymatic reactions. The NF is proposed to be used as food ingredient in the food category 'functional drinks', and as food supplement. The target population is the general population.

3.2 | Identity of the NF

The NF is a powder primarily consisting of monoglucosyl hesperidin (MGH) and unreacted hesperidin (flavonoid), which account in total for up to 92.8% (on dry basis) of the product (Table 1). In addition, the NF contains small amounts of diglucosyl

[1]On 15/04/2024 the applicant informed EFSA about the change in the name of the company to Nagase Viita Co., Ltd.

[2]Commission Implementing Regulation (EU) 2017/2469 of 20 December 2017 laying down administrative and scientific requirements for applications referred to in Article 10 of Regulation (EU) 2015/2283 of the European Parliament and of the Council on novel foods. OJ L 351, 30.12.2017, pp. 64–71.

hesperidin (di-GH) and maltooligosyl hesperidin contributing up to 2.8% and monoglycosyl flavonoids contributing up to 3.1% to the product. These flavonoids are produced concomitantly by glycosylation from hesperidin, containing hesperidin along with minor flavonoids as impurities. Free oligosaccharides derived from the raw material dextrin, e. g. β- and γ-cyclodextrins, are present in the NF at an amount of 0.8%.

The molecular structures of the main components of the NF, i.e. α-MGH and unreacted hesperidin, including the configuration of the anomeric carbons, were demonstrated by NMR spectroscopy (Figure 1).

TABLE 1 Chemical identity of α-monoglucosyl hesperidin (MGH).

Chemical substance	
Chemical (IUPAC) name	(2S)-7 -[(O-6-Deoxy-α-L-mannopyranosyl-(1→6)-O-[α-D-glucopyranosyl-(1→4)]-β-D-glucopyranosyl) oxy]-2,3-dihydro-5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-4H-1-benzopyran-4-one
Common name	glucosyl hesperidin
Synonyms	4G-α-D-glucopyranosyl-hesperidin; alpha-glucosyl-hesperidin; enzymatically modified hesperidin; monoglucosyl hesperidin
Abbreviations	α-GH; GH; MGH
Trade name	Hayashibara Hesperidin S; CitraPeak
CAS number	161713–86-6
Molecular formula	$C_{34}H_{44}O_{20}$
Molecular weight	772.70 Da

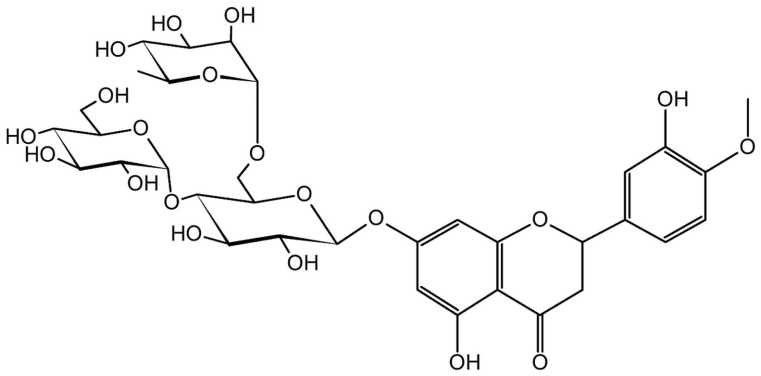

FIGURE 1 Chemical structure of monoglucosyl hesperidin (MGH).

3.3 | Production process

The production of the NF initiates with the dissolution of hesperidin (purity [redacted]) and dextrin in an alkaline solution. MGH is produced by two consecutive enzymatic reactions, after which the enzymes are inactivated by heat. The two enzymes used in the production of the NF have been assessed by the EFSA Panel on Food Contact Materials, Enzymes and Processing Aids (CEP), which concluded that both food enzymes do not give rise to safety concerns under the intended conditions of use (EFSA CEP Panel, 2023a, 2023b). After the inactivation of the enzymes, the solution undergoes a multistep purification process that includes filtration, chromatographic separation, intermediate concentration and decolourisation. The purified solution is then concentrated by evaporation, micro-filtrated and spray-dried to a moisture content ≤ 6.0%. The NF is stored in sealed aluminium laminated bags.

According to the information provided by the applicant, the NF is produced in line with ISO 9001:2015 and Hazard Analysis Critical Control Points (HACCP) principles.

The Panel considers that the production process is sufficiently described and does not raise safety concerns.

3.4 | Compositional data

The NF consists mainly of MGH with an average concentration of 77%. Unreacted hesperidin is present in the NF at an average of 14% and other hesperidin derivatives, i.e. di-GH (average concentration 0.9%) and maltooligosyl hesperidin (average concentration 1.9%), are also contained in the NF. Additionally, other glycosylated flavonoids, derived from the impurities of hesperidin (purity [redacted]), constitute a small percentage of the NF, i.e. monoglucosyl narirutin (1.2%), monoglucosyl diosmin (1.1%) and monoglucosyl neoponcirin (0.8%). Free oligosaccharides derived from the raw material dextrin (e.g. β-cyclodextrin, γ-cyclodextrin) are present at an average concentration of 0.8%.

To confirm that the manufacturing process is reproducible and adequate to produce a product with certain required characteristics on a commercial scale, the applicant provided analytical information for 10 independent batches of the NF (Table 2).

TABLE 2 Batch to batch analysis of the NF.

	Batch number										
Parameter (unit)	**#1**	**#2**	**#3**	**#4**	**#5**	**#6**	**#7**	**#8**	**#9**	**#10**	**Method of analysis**
Monoglucosyl hesperidin (MGH) (%)	75.9	76.0	77.8	77.3	76.9	76.7	76.5	76.9	76.5	76.4	HPLC-UV (in-house) for batches #1 - 5; HPLC-RI (in-house) for batches #6 - 10
Hesperidin (%)						14.4	14.3	14.5	15.0	14.5	HPLC-RI** (in-house)
Diglucosyl hesperidin (di-GH) (%)						0.28	0.23	0.26	0.23	0.31	HPLC-RI** (in-house)
Glycosyl ($n \geq 3$) hesperidin (%)						0.71	1.02	0.53	0.48	0.61	HPLC-RI** (in-house)
Monoglucosyl narirutin (%)						2.53	2.88	2.84	2.40	2.61	HPLC-RI** (in-house)
Narirutin (%)						0.51	0.57	0.68	0.52	0.68	HPLC-RI** (in-house)
Monoglucosyl neoponcirin (%)						2.83	3.09	2.57	2.74	3.16	HPLC-RI** (in-house)
Neoponcirin (%)						0.39	0.38	0.16	0.45	0.11	HPLC-RI** (in-house)
Monoglucosyl diosmin (%)						0.44	0.23	0.36	0.51	0.55	HPLC-RI** (in-house)
α-Cyclodextrin (%)						0.09	0.07	0.09	0.07	0.08	HPLC-RI (In-house)
β-Cyclodextrin (%)						0.45	0.48	0.53	0.47	0.46	HPLC-RI (In-house)
γ-Cyclodextrin (%)						0.23	0.12	0.23	0.16	0.25	HPLC-RI (In-house)
Other free saccharides (%)						0.12	0.00	0.16	0.20	0.14	HPLC-RI (In-house)
Total hesperidin (%)[3]	73.9	74.2	76.1	75.7	75.6						Calculation expressed as hesperidin based on HPLC-UV (In-house)
Loss on drying (%)	2.6	2.3	2.4	2.3	2.8						Gravimetry ('Enzymatically Modified Hesperidin' monograph in Japan's Specifications and Standards for Food Additives)
Residue on ignition (%)	0.1	0.1	0.0	0.0	0.0						Gravimetry
pH	5.7	5.7	5.7	5.6	5.7						Electrochemical method (Japanese Industrial Standards 'Z 8802')
Heavy metals											
Lead (mg/kg)	≤ 2*	≤ 2*	≤ 2*	≤ 2*	≤ 2*						Flame atomic absorption spectrometry (Standard Methods of Analysis for Hygienic Chemists: with Commentary Methods of Analysis in Health Science (2015))
Arsenic (mg/kg)	≤ 1.5*	≤ 1.5*	≤ 1.5*	≤ 1.5*	≤ 1.5*						Cold vapour atomic absorption spectrometry (Standard Methods of Analysis for Hygienic Chemists: with Commentary Methods of Analysis in Health Science (2015))

Abbreviations: HPLC-RI, high-performance liquid chromatography refractive index; HPLC-UV, high-performance liquid chromatography ultraviolet detection.

*LOQ, limit of quantification.

**Confirmation of molecular weight by LC-MS (liquid-chromatography-mass spectrometry).

Following a request from EFSA, the applicant provided analytical data on residual ethanol in five batches of the NF by headspace gas chromatography, different from the batches presented in Table 2 (batches #11–15), and ethanol was found at < 0.01% in four batches and at 0.03% in one batch. In addition, the applicant was requested to provide analytical data on cadmium and mercury in the NF. These two metals were analysed in five batches (#4 and #5 and three not included in Table 2) by atomic absorption spectrometry. All concentrations were below the LOQ (0.01 mg/kg).

The applicant also presented data on microbiological parameters in five batches on the NF (Table 3).

[3]Hesperidin (%) = $(A_{TH}/A_S) \times (W_S/W_T) \times (100/250) \times 0.790 \times 25 \times 100$. A_{TH}: The peak area of hesperidin in the test solution. A_S: The peak area of MGH in the standard solution. W_S: The amount of MGH standard (g). W_T: The amount of sample (g). 0.790: Molecular weight of hesperidin (610.56)/ Molecular weight of MGH (772.70).

TABLE 3 Microbiological parameters in five batches of the NF.

	Number of batch										
Parameter (unit)	**#1**	**#2**	**#3**	**#4**	**#5**	**#11**	**#12**	**#13**	**#14**	**#15**	**Method of analysis**
Total aerobic microbial count (CFU/g)	< 1*	< 1*	1	< 1*	< 1*						Japanese Pharmacopeia 18th edition (equivalent to USP <2021>)
Coliform organisms/g	ND	ND	ND	ND	ND						Pharmaceutical Society of Japan
***Escherichia coli*/10 g**	ND	ND									Japanese Pharmacopeia 18th edition
Yeast and moulds (CFU/g)						< 1*	< 1*	< 1*	< 1*	< 1*	Japanese Pharmacopeia 18th edition
***Salmonella*/10 g**						ND	ND	ND	ND	ND	Japanese Pharmacopeia 18th edition
Water activity						0.22	0.25	0.26	0.12	0.11	Japanese Pharmacopeia 18th edition

Abbreviations: CFU, colony forming units; ND, not detected; USP, United States Pharmacopeia.

*LOQ: limit of quantification.

Laboratories that conducted the analyses presented in the application are accredited in accordance with the recognised International Standard ISO/IEC 17025:2017.

The water solubility of the NF was measured at 5, 10 and 25°C and was reported as > 123, > 153 and > 197 g/100 g water, respectively. The water solubility of hesperidin is reported to be around 2 mg/100 g.[4]

The Panel considers that the information provided on the composition is sufficient for characterising the NF.

3.4.1 | Stability

The applicant performed stability tests with independently produced batches of the NF. A test with five batches was carried out at normal storage conditions of 20–30°C and humidity was not controlled for a period of 42 months and one batch was tested at 25°C and 60% relative humidity (RH) for 40 months. Moreover, the stability of the NF was tested under accelerated conditions at 40°C and 75% RH for 7 months (two batches) and 3 months (one batch). The batches were analysed for physicochemical parameters including MGH, total hesperidin, loss on drying, pH and microbiological parameters (total aerobic microbial count and coliforms). None of the parameters tested changed significantly during the course of the studies.

The stability of the NF was also tested in aqueous solutions (final concentration 1% w/v) under different pH (i.e. pH 3, pH 4, pH 5, pH 6, pH 7) at 5, 25 and 55°C up to 4 weeks. After storage, the recovery rates of MGH and hesperidin were determined in each solution at baseline, and after 1 week, 2 weeks and 4 weeks. MGH and hesperidin were stable in aqueous solutions under the conditions tested, except for 4-week storage at 55°C, pH 7.0. After the 4-week storage at 55°C, pH 7, MGH and hesperidin were slightly reduced (recovery rate 88%).

Additionally, long-term stability was tested in aqueous solutions (final concentration 0.1% w/v) at pH 3, pH 5 and pH 7 at 25°C for up to 12 months in one batch. The recovery rate of MGH was stable in aqueous solutions under the tested conditions for up to 12 months. Hesperidin exhibited a 90%–100% recovery rate in aqueous solutions after 12 months of storage.

The stability of one batch of the NF was also tested in boiling water (final concentration 1% w/v) for 30, 60 and 90 minutes under various pH (i.e. pH 3, pH 4, pH 5, pH 6, pH 7). Recovery rates of MGH and hesperidin did not change during the course of the study.

The stability of one batch of the NF was also tested in the dough of a pound cake baked in an oven for 20 and 45 minutes at 160°C. The recovery rates of MGH and the glycosylation rate (expressed as the ratio of the peak area of MGH in the test solution to the sum of the peak areas of MGH and hesperidin in the test solution) were calculated 2 hours after dough preparation, after baking for 20 min at 160°C and after baking for 45 min at 160°C. MGH content was decreased by baking at 160°C (to 94% in 20 min and 87% in 45 min) whereas the glycosylation rate remained stable.

Finally, the applicant also tested the photostability of one batch of the NF in aqueous solution at pH 3, pH 3.5 and pH 4 for two sample concentrations (0.1% and 0.5% w/v) using irradiation at 11,000 lux at 40°C. Recovery rates of MGH and hesperidin were calculated at baseline, and after 4, 8 and 12 h and the rates did not change over the course of the study except for the sample of 0.5% w/v at pH 3. The recovery rates of MGH in this case decreased slightly to 98% and 96% and for hesperidin to 99% and 95% at 8 and 12 h, respectively.

The Panel considers that the data provided sufficient information with respect to the stability of the NF.

3.5 | Specifications

The specifications of the NF are indicated in Table 4.

[4]Calculated using Advanced Chemistry Development (ACD/Labs) Software V11.02 (© 1994–2024 ACD/Labs).

TABLE 4 Specifications of the NF.

Description: A pale yellow to yellow-brown powder produced enzymatically from hesperidin and dextrin	
Parameter	Specification
Monoglucosyl hesperidin (MGH) (dry basis)	75.0%–85.0%
Hesperidin (dry basis)	10%–20%
Loss on drying	≤ 6%
Residue on ignition	≤ 2%
Heavy metals	
Lead	≤ 2 mg/kg
Arsenic	≤ 1.5 mg/kg
Microbiological	
TAMC	≤ 100 CFU/g
Total coliforms	Not detected in 10 g
Salmonella spp.	Not detected in 25 g
Yeast and moulds	< 100 CFU/g

Abbreviations: CFU, colony forming units; TAMC, total aerobic microbial count.

The Panel considers that the information provided on the specifications of the NF is sufficient and does not raise safety concerns.

3.6 | History of use of the NF and/or of its source

3.6.1 | History of use of the source

Hesperidin, which is used as raw material for the production of the NF, is isolated from the peels, juice, or seeds of citrus fruits. Hesperidin is a flavonoid that is naturally present in various Citrus species (Garg et al., 2001), with an estimated daily intake of up to 8 mg/kg body weight (bw) in adults (Koch et al., 2013). It has a history of use in the EU in food supplements prior to 15 May 1997 at various proposed daily doses, but usually not exceeding 600 mg/day.

Dextrin, another compound used as raw material for the production of the NF, is a mixture of glucose polymers which is commonly found in food.

3.6.2 | History of use of the NF

The NF is listed in the List of Existing Food Additives in Japan and marketed since 1998 as a food ingredient in a variety of food categories and use levels (JMHLW, 2018; JMOH, 1996a). Approximately 20 tons of the NF are sold annually in Japan. The NF is monographed in the South Korean Food Additives Code, but the applicant notes that the product is prepared with the use of different enzymes (MFDS, 2019). The NF is also listed on the Food Ingredients List under the name of 'α-glycosyl hesperidin' in Taiwan (Taiwan FDA, 2017). In the US, the NF is marketed under notified GRAS and no question letter has been issued (GRN No. 901, 2019).

3.7 | Proposed uses and use levels and anticipated intake

3.7.1 | Target population

The target population proposed by the applicant is the general population, except for food supplements for which the proposed target population is children from 1 year onwards and adults.

3.7.2 | Proposed uses and use levels

Initially, the proposed uses of the NF included several hot beverages, non-alcoholic beverages, confectionery and food supplements as defined in Directive 2002/46/EC. After the additional data requests, the applicant adjusted the proposed uses of the NF to be used as an ingredient in the food category 'functional drinks' (based on EFSA FoodEx2[5] hierarchy). In

[5]FoodEx2 is an EFSA standardised food classification and description system (https://www.efsa.europa.eu/en/data/data-standardisation).

addition, the NF is proposed to be used in food supplements, as defined in Directive 2002/46/EC, at different maximum daily intakes, depending on the population group. These proposed uses and maximum use levels (mg NF/L or mg NF/day) are reported in Table 5.

TABLE 5 Food categories and maximum use levels intended by the applicant.

FoodEx2 level	FoodEx2 code	Food category	Max use level
		Food supplements	200 mg/day for general population older than 10 years 115 mg/day for children between 3 and 10 years of age 60 mg/day for young children between 1 and 3 years of age
L3	A03FZ	Functional drinks[a]	525 mg/L

[a]This food category includes: energy drinks, isotonic and sport drinks and fermented functional drinks (i.e. 'fermented non-alcoholic drinks with exclusion of dairy fermented drinks)'. The use of this code does not indicate a health claim under Regulation (EC) No 1924/2006.

3.7.3 | Anticipated intake of the NF

EFSA performed an intake assessment of the anticipated daily intake of the NF based on the applicant's proposed uses and maximum proposed use levels (Table 5), using individual data from the EFSA Comprehensive European Food Consumption Database (EFSA, 2011). The highest 95th percentiles anticipated daily intake of the NF, among the EU dietary surveys, are presented in Table 6 [on a mg/kg bw basis] and total daily intake was calculated taking also into consideration the proposed use levels of the NF as food supplement.

The estimated daily intake of the NF for each population group from each EU dietary survey is available in the Excel file annexed to this scientific opinion (under Supporting information).

TABLE 6 Total intake of the NF resulting from its uses as an ingredient and as a food supplement.

Population group	Age (years)	Body weight[a] (kg)	Highest[b] mean intake from the NF used as an ingredient (mg/kg bw per day)	Highest[c] P95 intake from the NF used as an ingredient (mg/kg bw per day)	Intake from the NF used as a food supplement (mg/kg bw per day)[d]	Total intake[e] (mg/kg bw per day)	Total intake (mg/day)
Infants	< 1	5	NA	NA	–	–	–
Toddlers	1–< 3	12	0	0	5	5	60
Other children	3–< 10	23.1	0.1	0	5	5.1	118
Adolescents	10–< 14	43.4	0.2	1.3	4.6	5.9	256
Adolescents	14–< 18	61.3	0.2	1.3	3. 3	4.9	300
Adults	≥ 18	70	0.3[f]	2.3[f]	2.9	5.2	364

Abbreviation: NA, not available.

[a]Default and average body weights are defined in EFSA Scientific committee (2012).

[b]Intakes are assessed for all EU dietary surveys available in the food comprehensive database on 15/05/2024. The highest mean observed among all EU surveys is reported in this column.

[c]Intakes are assessed for all EU dietary surveys available in the food comprehensive database on 15/05/2024. The highest P95 observed among all surveys is reported in this column (P95 calculated based on less than 60 individuals are not considered).

[d]Intake in 'mg/kg bw per day' are calculated by considering the use levels in 'mg/day' and default body weights defined in EFSA Scientific Committee (2012).

[e]Total intake is the sum of the intake from NF ingredient use (highest mean or highest P95, if available) and from the NF used as a food supplement, for each population group.

[f]Intakes are assessed separately for adults (18–64 years), elderly (65–74 years) and very elderly (≥ 75 years); the maximum intake among these three sub-populations is reported here.

The Panel notes that no data are available for infants from the EFSA Comprehensive European Food Consumption Database regarding the consumption of 'functional drinks'. Furthermore, the Panel notes that the highest total daily intake of the NF is up to 5.9 mg/kg bw for adolescents (10–14 years of age).

3.8 | Absorption, distribution, metabolism and excretion (ADME)

Animal studies have investigated the bioavailability and kinetics of MGH in comparison to hesperidin after single-dose oral administration of each of these test substances in equimolar doses of 1 mmol/kg bw (Yamada, Arai, et al., 2006) or 0.5 mmol/kg bw (Mitsuzumi et al., 2006, 2008). Tested substances and their metabolites were then measured at different time points

in serum and/or different organs, using HPLC. Both MGH and hesperidin, when orally administered, reach the small intestine unmodified. A major portion of MGH is hydrolysed to hesperidin by the α-glucosidase in the jejunum (Mitsuzumi et al., 2008, unpublished). After this initial step, both substances follow (or seem to follow) the same metabolic pathway including further hydrolysis into rutinose and hesperetin, mainly by bacterial β-glucosidase and/or α-rhamnosidase in the colon (Amaretti et al., 2015; Yamada, Arai, et al., 2006). The Panel notes that hydrolysis of MGH and hesperidin seems to be complete, as no traces are found in faeces, urine or in the investigated parts of the GI-tract and organs (stomach, jejunum, ileum, caecum, liver and kidneys) (Yamada, Arai, et al., 2006; Yamada, Tanabe, et al., 2006, unpublished). The aglycone (hesperetin) is taken up by enterocytes, which are the main site of modification by methylation, sulfation, glucuronidation or a combination of these processes. Conjugation can also take place in the liver and only conjugated forms (e.g. hesperetin-glucuronide) enter the systemic circulation unless the MGH and hesperidin are administered in high amounts (data from animal studies only) when small amounts of hesperetin can be found in the bloodstream (Matsumoto et al., 2019). The Panel notes that the area under the curve ($AUC_{0\text{-}27h}$) for conjugated hesperetin in serum is found to be approximately 3.7-fold higher when administered in an equimolar dose of MGH and dissolved in water as compared to hesperidin, with an earlier T_{max} and higher C_{max}. Also, urinary excretion of both hesperetin and conjugated forms was higher in rats of the MGH group (Yamada, Arai, et al., 2006), indicative of a higher absorption. However, when administered with the emulsifier sodium carboxymethyl cellulose (CMC-Na), there seems to be no difference in these parameters (Mitsuzumi et al., 2006, unpublished). The Panel notes that in all the animal studies, an isolated and purified hesperidin was used as a comparator, which may not represent the kinetic profile of hesperidin when it is naturally present in a food matrix (e.g. citrus fruit).

An ADME study in healthy humans (5 men and 5 women) was conducted by Yamashita et al. (2008, unpublished) to investigate the bioavailability and kinetics of MGH and its metabolites. In Test 1, a single dose of 500 mg of glucosyl-hesperidin in 50 mL of water was administered and blood samples were taken up to 25h post-administration. Test 2, following a minimum 1-week washout period, involved the daily administration of the same dose for 2 weeks, with weekly blood samples taken 8h post-administration (set due to T_{max}), and 1 week after administration had stopped. The results of Test 1 indicated high variability among participants in terms of T_{max} (9.22 ± 6 h), C_{max} (0.750 ± 0.699 μmol/L) and $AUC_{0\text{-}25h}$ (6.02 ± 3.76 μmol/L · h) for conjugated hesperetin. Both tests indicated that MGH does not enter the systemic circulation, but is hydrolysed to hesperetin, which is then conjugated in the digestive tract with glucuronic acid to hesperetin-glucuronide, the only metabolite measured in the bloodstream. The results of both tests further showed that the blood concentrations of this metabolite remained relatively constant during Test 2 and were cleared from the circulation 1 week post-administration. During Test 2, two subjects reported abdominal symptoms that were judged not to be related to the test substance.

3.9 | Nutritional information

The NF is mainly composed of MGH (75%–85%) and hesperidin (10%–20%), and small amounts of other flavonoids and oligosaccharides (see Section 3.4, Compositional data). The applicant provided analytical data for nutritional parameters in two batches of the NF. The water content was on average at 2.5% and protein, lipid and ash were all < 0.1% whole weight.

The Panel considers that taking into account the composition of the NF and the proposed conditions of use, consumption of the NF is not nutritionally disadvantageous.

3.10 | Toxicological information

The applicant provided eight toxicological studies with the NF, with a test substance similar to it, but produced with a different production process and/or deviating from the current NF specifications (see Sections 3.10.1 Genotoxicity and 3.10.3 Reproductive and developmental toxicity). Submitted studies were conducted in compliance with OECD principles of GLP (OECD, 1998a) and in accordance with the test guidelines (TG) No 471, 473 and 487 (OECD, 2010) or according to Guidelines for designation of food additives and for revision of standards for use of food additives Chapter V 'The recommended methods for safety studies', Ministry of Health, Labor and Welfare, Notification No. 29, March 22, 1996, Japan (JMOH, 1996b). Key information about these studies are listed in Table 7.

TABLE 7 List of toxicological studies with the NF or with a test substance similar to it.

Reference	Type of study	Test system	Dose
Unpublished study report (1997)[a]	Bacterial reverse mutation test (GLP, OECD TG No. 471 from 1983)	Experiment I: S. typhimurium TA98, TA100, TA102, TA1535 and TA1537 and *E. coli* WP2 uvrA Experiment II: S. typhimurium TA98, TA100, TA1535 and TA1537 and *E. coli* WP2 uvrA	Experiment I: Up to 5000 μg/plate (plate incorporation test), absence and presence of S9-mix Experiment II: Up to 5000 μg/plate (pre-incubation test), absence and presence of S9-mix

(Continues)

TABLE 7 (Continued)

Reference	Type of study	Test system	Dose
Unpublished study report (2023)	Bacterial reverse mutation test (GLP, OECD TG No. 471 from 1997a)	S. typhimurium TA98, TA100, TA1535 and TA1537 and *E. coli* WP2 uvrA	Up to 5000 μg/plate (pre-incubation test), absence and presence of S9-mix
Unpublished study report (2022)	In vitro micronucleus test (GLP, OECD TG No. 487, 2010)	TK6 (derived from human spleen lymphoblast)	Short-term exposure (3 h): up to 2000 μg/mL in presence and absence of S9-mix Continuous exposure (24 h): up to 2000 μg/mL in the absence of S9-mix
Unpublished study report (2007a)	In vitro chromosome aberration test [GLP, 'Guidelines for designation of food additives and for revision of standards for use of food additives', Chapter V 'The recommended methods for safety studies', Ministry of Health, Labor and Welfare, Notification No. 29, March 22, 1996, Japan, (JMOH, 1996b)]	Chinese hamster lung fibroglast cells (CHL/IU)	Short-term exposure (6h): up to 5000 μg/mL in presence and absence of S9-mix Continuous exposure (24 h): up to 5000 μg/mL in the absence of S9-mix
Unpublished study report (2006)	In vivo micronucleus test [GLP, 'Guidelines for designation of food additives and for revision of standards for use of food additives', Chapter V 'The recommended methods for safety studies', Ministry of Health, Labor and Welfare, Notification No. 29, March 22, 1996, Japan (JMOH, 1996b)]	Mouse CrljBgi:CDI (ICR), SPF strain (bone marrow cells)	Control: 0 (% distilled water); 500, 1000 and 2000 mg/kg
Unpublished study report (2005a)[b]	28-day oral toxicity study (GLP)	Rats, HanBrl:WIST(SPF)	0, 100, 2000 and 15,000 mg/kg diet, i.e. 0, 8, 157 and 1206 mg/kg bw for males, and 0, 8.5, 171 and 1280 mg/kg bw per day for females
Unpublished study report (2005b)[b]	90-day repeated dose oral toxicity study (GLP, OECD TG No. 408)	Rats, HanRcc:WIST (SPF)	0, 4500, 15,000 and 50,000 mg/kg diet, i.e. 0, 279, 927 and 3084 kg/bw per day for males, and 0, 322, 1064 and 3428 kg/bw per day for females
Unpublished study report (2007b)[c]	Pre-natal developmental toxicity study [GLP, 'Guidelines for designation of food additives and for revision of standards for use of food additives', Chapter V 'The recommended methods for safety studies', Ministry of Health, Labor and Welfare, Notification No. 29, March 22, 1996, Japan (JMOH, 1996b)]	Rats, Crl:CD(SD)	0, 100, 300 and 1000 mg/kg bw per day

[a]Study not conducted with the NF.

[b]Original CoAs for test substance were not available in the original study reports, thus the applicant reissued them based on the raw data, including the production and analysis dates of the production lots used.

[c]Content of monoglucosyl-hesperidin in the test substance was 70.2%.

3.10.1 | Genotoxicity

Initially, to demonstrate the absence of mutagenic effects of the NF, the applicant provided the bacterial reverse mutation test (Unpublished study, 1997), conducted with the test substance (claimed by the applicant to be of 'cosmetic grade') which the Panel did not consider as representative of the NF due to the use of an additional enzyme (α-L-rhamnoside rhamnohydrolase) in the production process and due to the values of several analytical parameters which did not correspond to the specifications of the NF. Therefore, the applicant was requested to conduct a new bacterial reverse mutation test using the NF.

As requested, the bacterial reverse mutation test (Unpublished study, 2023) was performed according to OECD Test Guideline No. 471 (OECD, 1997a) and in compliance with the principles of Good Laboratory Practice (GLP). Using S. typhimurium tester strains TA98, TA100, TA1535, TA1537 and *E. coli* WP2uvrA and two independent pre-incubation tests, the NF was tested at dose levels of 0 (vehicle control: sterilised distilled water), 313, 625, 1250, 2500 and 5000 μg/plate in the absence and presence of a metabolic activation system (S9-mix). There were no dose-related increases in the number of revertant colonies, and all increases were below 2-fold as compared to the negative control. Positive controls were functional, and all results were within the historical control data of the laboratory.

The applicant initially submitted another study, an in vivo micronucleus (MN) assay (Unpublished study, 2006) (GLP statement provided), which was conducted according to the 'Guidelines for designation of food additives and for revision of standards for use of food additives', Chapter V 'The recommended methods for safety studies', Ministry of Health, Labor and Welfare, Notification No. 29, March 22, 1996, Japan (JMOH, 1996b) similar to the OECD TG No. 474 (OECD, 2014). Bone marrow cells from five male mice per group [CrljBgi:CDI (ICR)] were tested for the formation of micronuclei. In the preliminary toxicity test, mice were exposed to the NF by gavage at the dose levels of 0 (distilled water), 125, 250, 500, 1000 and 2000 mg/kg bw. The results showed that, at the highest dose tested, there were no relevant changes in the general condition of the mice and no deaths occurred. In the micronucleus induction frequency test which lasted for 24, 48 and 72 h, mice were exposed to 2000 mg/kg bw. No increase in micronucleus count was observed. Finally, in the full micronucleus test (24 h), mice were exposed to 0 (distilled water), 500, 1000 and 2000 mg NF/kg bw, while the positive control was mitomycin C (intraperitoneally). No signs of toxicity and no changes in the body weight were observed. None of the dose-groups showed significant increases in the frequency of micronucleus formation compared to the control. The ratio of polychromatic erythrocytes to the total erythrocyte count showed no significant difference between the various treatment groups and the control. The Panel notes limitations in the study since no historical control data were provided and only 2000 cells were scored for the incidence of micronuclei, instead of the 4000 required by the corresponding OECD TG 474. The Panel also notes that this in vivo MN test does not provide evidence of bone marrow exposure. Therefore, in line with the requirements set in the EFSA Novel Food Guidance (EFSA NDA Panel, 2016) and the Guidance of the EFSA Scientific Committee (2011) on genotoxicity testing strategies applicable to food and feed safety, EFSA recommended an in vitro MN study (OECD TG No. 487, 2010) in order to have a completed first tier (i.e. in vitro) genotoxicity testing battery.

Following EFSA's request, the applicant provided an in vitro MN test in human TK6 lymphoblastoid cells (Unpublished study, 2022), conducted in compliance with the principles of GLP and according to the OECD TG No. 487 (2010). The dose-finding test was conducted at doses up to 2000 µg/mL and the results showed that the 50% cell-growth inhibition concentration was 746.7 µg/mL in the 24-h assay, while in the short-term assay (both with and without S9-mix), no cell growth inhibition was observed. In the main test, TK6 cells were exposed to 0 (distilled water), 250, 500, 1000 and 2000 µg NF/mL in the presence and absence of S9-mix (short-term exposure of 3 h) or to 0 (distilled water), 250, 375, 500, 600, 700, 800, 900, 1000, 1500 and 2000 µg NF/mL in the absence of S9-mix (continuous/long-term exposure of 24 h). Cytotoxicity test conducted in the main test showed that RPD (relative population doubling) was more than 50% at all concentrations in the presence and absence of S9-mix and that RPD was 48.1% at 800 µg/mL during the continuous exposure. Thus, for the continuous duration of exposure, the highest dose subject to microscopic observations was 800 µg/mL. Microscopic observations showed that the count of cells with micronuclei, across all dose levels and treatment conditions, fell within the historical control range of the negative control of the test facility.

The applicant initially also submitted an in vitro chromosome aberration test with the NF, using rodent lung fibroblast cells (CHL/IU) (Unpublished study report, 2007a). The test was conducted in compliance with the principles of GLP and according to the 'Guidelines for designation of food additives and for revision of standards for use of food additives', Chapter V 'The recommended methods for safety studies', Ministry of Health, Labor and Welfare, Notification No. 29, March 22, 1996, Japan (JMOH, 1996b). In the cell-growth inhibition test, the NF was tested in doses of 0 (distilled water), 4.9, 9.8, 19.6, 39.1, 78.2, 156.3, 312.5, 625, 1250, 2500 and 5000 µg/mL, both in the presence and absence of S9-mix. No cytotoxicity was observed up to the highest dose tested. In the main experiment, the NF was tested in doses of 0 (distilled water), 1250, 2500 and 5000 in the presence and absence of S9-mix (short-term exposure, 6h) and in the absence of S9-mix (continuous exposure, 24 h). No chromosomal structural or numeric aberrations (polyploidy) were induced by the NF at any of the dose levels, during either short-term or continuous exposure. The Panel notes that only 200 cells, instead of 300 as required in the corresponding and current OECD TG No. 473 were scored.

Overall, taking into account that the requested in vitro studies, which were conducted with the NF and belong to the first tier of the recommended genotoxicity testing strategy (EFSA Scientific Committee, 2011), i.e. the bacterial reverse mutation test (Unpublished study, 2023) and the MN test (Unpublished study, 2022), provided negative results, the Panel considers that there are no concerns regarding genotoxicity.

3.10.2 | Subacute and subchronic toxicity

The applicant provided a 28-day oral dose-range finding study (Unpublished study report, 2005a) conducted in compliance with GLP and claimed to be in accordance with OECD TG No. 407 (OECD, 1995). Rats [HanBrl: Wistar (WISTSPF), 5 animals/sex per group] were administered the NF, i.e. the test substance matching the key specification parameters. The test substance was administered via feed for 28 days in amounts of 0, 100, 2000 and 15,000 mg/kg diet, corresponding to 0, 8, 157, 1206 mg/kg bw per day for males and 0, 8.5, 171 and 1280 mg/kg bw per day for females, based on feed consumption. All animals survived the scheduled study period. No clinical signs, no significant effects on body weight or body weight gain, no changes in feed consumption, and no test item-related gross lesions were observed during the study duration or at necropsy, compared to the control animals. Haematological and clinical chemistry parameters were not investigated which is not in accordance with the OECD TG 407 (1995).

In the 90-day oral toxicity study (Unpublished study report, 2005b), the NF, i.e. the test substance matching the key specification parameters, was administered at the doses of 0, 4500, 15,000 and 50,000 mg/kg feed, corresponding to 0, 279, 927, 3084 mg/kg bw per day for males and 0, 322, 1064, and 3428 mg/kg bw per day for females, based on feed consumption.

The study was conducted in compliance with GLP and in accordance with OECD TG No. 408 (OECD, 1998b). Ten animals/sex [rats, HanRcc:WIST(SPF)] per group were used. No mortalities or clinical signs, and no effects on food consumption and body weight were reported. There were no test item-related macroscopic or microscopic findings except for centrilobular hepatocellular hypertrophy in two males of the high-dose group. The following haematological parameters were increased in a dose-dependent manner, reaching statistical significance ($p < 0.05$ or $p < 0.01$) in the high-dose group in comparison to the control group of animals: reticulocytes in males (+17%), lymphocytes in females (+43%), large unstained cells in females (+67%) and white blood cells in females (+35%). Furthermore, clinical chemistry parameters such as plasma levels of sodium and phosphorus were also statistically significantly increased in the high-dose group. However, all the values regarding haematological and clinical chemistry parameters were within the provided historical control range of the test facility. Organ weights were all similar to the controls, except for absolute testes weight, which decreased significantly (−9.1%) in both the low- and high-dose group and the same effect was observed for the relative to brain weight (Appendix A). Upon EFSA's request, the applicant was unable to provide either the data on thyroid weight or the historical control ranges for organ weights. The Panel notes a pattern of changes in several of the previously mentioned parameters within the current study which raises concern of adverse effects observed at the highest dose tested. Therefore, the Panel considers the mid-dose (~ 1000 mg/kg bw per day) as NOAEL from this study.

The applicant also provided several repeated-dose toxicity studies found in the literature. Kurata et al. (1990) and Kawabe et al. (1993) used methyl-hesperidin; therefore, the Panel does not consider these studies appropriate for the safety assessment of the NF, due to the use of different test substances.

Li et al. (2019) conducted a 90-day study using hesperidin (73% purity), a metabolite of the NF, which was isolated from a methanolic extract of dried orange peel. The highest dose tested (1000 mg/kg bw per day) was considered as LOAEL by the study authors due to several alterations in body and organ weights, haematology, clinical chemistry and tissue histopathology. The Panel considers these findings as supportive to the findings reported in the 90-day study with the NF, while acknowledging the lower purity of the tested substance compared to hesperidin ([redacted] purity) used in the production of the NF.

3.10.3 | Reproductive and developmental toxicity

A pre-natal developmental toxicity study (Unpublished study report, 2007b), conducted in compliance with GLP and in accordance with Guideline for Designation of Food Additives and for Revision of Standards for Use of Food Additives (EIKA, No. 28, March 22, 1996, Japan), similar to OECD TG No. 414, was provided by the applicant. Twenty mated female rats [Crl:CD(SD)] per group were administered the test substance similar to the NF (containing 70.2% of MGH and 14.3% of 'unknown') by gavage at doses of 0 (water), 100, 300 and 1000 mg/kg bw per day from day 6 to day 17 of gestation and were sacrificed at day 20 of gestation. There were no adverse findings in this study and the Panel agrees with the authors' conclusion that the maternal and fetal developmental NOAELs of the test substance were both 1000 mg/kg bw per day, the highest dose tested.

3.10.4 | Human data

The applicant provided 11 publications reporting on human intervention (efficacy) studies and using GH or MGH as reported test substance, of which three (Miwa et al., 2004, 2005; Takumi et al., 2010) did not assess safety-related endpoints and will not be discussed further. The key characteristics of the remaining eight studies are summarised in Appendix B.

The test food was provided in tablets either alone ($n = 1$) or in combination with caffeine ($n = 1$); dissolved in tea ($n = 2$), water ($n = 2$) or soy sauce ($n = 1$); or in an unspecified food matrix ($n = 1$). Only three studies used a test food provided by the applicant (Kozuma et al., 2007; Nakagawa et al., 2008; Ohara et al., 2016). Doses of GH/MGH ranged from 35 mg/day to 3 g/day and the intervention lasted between 4 and 12 weeks. The number of subjects per study assigned to GH/MGH ranged between 10 (in the study using 3 g/day for 12 weeks; Kometani et al., 2008) and 81. All the studies assessed clinical chemistry, and all but one haematological parameters (WBC, RBC, haematocrit, haemoglobin, platelet count, MCV, MCH, MCHC, lymphocytes, monocytes, neutrophils, eosinophils and basophils) as part of the safety evaluation. None of the studies reported adverse effects on any of these endpoints, or adverse events that could be related to the consumption of GH/MGH.

The Panel notes the limitations of these human intervention studies in assessing the safety of the NF, e.g. only three studies were designed to assess both efficacy and safety (Kozuma et al., 2007; Tanaka et al., 2010; Yuasa et al., 2005), unclear compliance of the test foods with the specifications of the novel food, since the supplier of the test substance was not always the applicant or not reported. The Panel also notes that no adverse effects on haematology or clinical chemistry, and no adverse events related to the consumption of GH/MGH, have been observed at doses up to 3 g/day for up to 12 weeks.

3.11 | Allergenicity

The Panel considers that, owing to the nature of the source material used for the production of the NF and the low protein content, the NF is unlikely to trigger allergic reactions in the target population under the proposed conditions of use.

4 | DISCUSSION

The NF, which is the subject of the application, is a pale yellow to yellow-brown powder consisting of MGH (75%–85% on dry matter basis) produced from hesperidin and dextrin by enzymatic reactions.

The NF is proposed to be used as food ingredient in specific drinks, and as food supplement resulting in combined intake estimates of up to 5.9 mg/kg bw per day in adolescents. The target population is the general population, except for food supplements for which the proposed target population is children from 1 year onwards and adults.

Taking into account the composition of the NF and the proposed conditions of use, consumption of the NF is not nutritionally disadvantageous.

A pattern of changes was observed for several parameters (centrilobular hepatocellular hypertrophy in males, and haematological and clinical chemistry parameters) at the highest dose tested in the provided subchronic toxicity study (Unpublished study report, 2005b). Therefore, the Panel considers the NOAEL at the mid-dose group, i.e. ~1000 mg/kg bw per day. By applying a default uncertainty factor of 200 [10 (interspecies variability) × 10 (intraspecies variability) × 2 (extrapolation from subchronic to chronic study duration in rodents)], the resulting intake for humans would be 5 mg/kg bw per day.

Hesperidin, the primary metabolite of MGH, is a naturally occurring flavonoid (e.g. in citrus fruits and products thereof), so that the total dietary intake of hesperidin (from the NF and the background diet) contributes to the systemic exposure to hesperidin metabolites.

The Panel notes that hesperidin has a history of use in the EU from dietary sources and food supplements in amounts which would exceed the daily intake of hesperidin from the NF (see Section 3.6). The Panel also notes that the available human intervention studies did not report clinically relevant changes in haematological or clinical chemistry parameters following the administration of GH/MGH at supplemental doses of up to 3 g/day for 12 weeks (see Section 3.10.4). Overall, the Panel considers that the margin of exposure (~200) between the intake of the NF at the proposed use and use levels and the NOAEL from the 90-day study is sufficient.

5 | CONCLUSIONS

The Panel concludes that the NF, glucosyl hesperidin, is safe for the target population at the proposed uses and use levels.

5.1 | Protection of proprietary data in accordance with article 26 of regulation (EU) 2015/2283

The Panel could not have reached the conclusion on the safety of the NF under the proposed conditions of use without the data claimed as proprietary by the applicant: (i) detailed description of the production process; (ii) composition and stability of the NF; and (iii) toxicological information including in vitro genotoxicity studies, 90-day subchronic toxicity study and teratogenicity study.

6 | STEPS TAKEN BY EFSA

1. On 23/09/2021 EFSA received a letter from the European Commission with the request for a scientific opinion on glucosyl hesperidin as a novel food. Ref. Ares(2021)5809066–23/09/2021.
2. On 23/09/2021, a valid application on glucosyl hesperidin, which was submitted by the company Hayashibara Co, Ltd., was made available to EFSA by the European Commission through the Commission e-submission portal (NF 2021/2455) and the scientific evaluation procedure was initiated.
3. On 15/04/2024 the applicant informed EFSA about the change in the name of the company to Nagase Viita Co., Ltd.
4. On 26/01/2022, 16/11/2022, 20/02/2023, 28/03/2023, 29/11/2023, 06/05/2024 and 31/05/2024 EFSA requested the applicant to provide additional information to accompany the application or to clarify previously submitted data and the scientific evaluation was suspended.
5. On 25/10/2022, 31/01/2023, 08/03/2023, 22/08/2023, 15/04/2024, 15/05/2024 and 03/06/2024 additional information or clarification to the previously submitted data was provided by the applicant through the Commission e-submission portal and the scientific evaluation was restarted.
6. During its meeting on 26/06/2024, the NDA Panel, having evaluated the data, adopted a scientific opinion on the safety of glucosyl hesperidin as a NF pursuant to Regulation (EU) 2015/2283.

ABBREVIATIONS

ADME	absorption, distribution, metabolism and excretion
AUC	area under the curve
bw	body weight
CAS	chemical abstracts service

CDI	In table 7 (page 10)
CEP	EFSA Panel on Food Contact Materials, Enzymes and Processing Aids
CFU	colony forming units
CHL/IU	Chinese hamster lung cell line
C_{max}	maximum concentration
CMC-Na	sodium carboxymethylcellulose
CoA	certificate of analysis
di-GH	diglucosyl hesperidin
FDA	Food and Drug Administration
GH	glucosyl hesperidin
GI	gastrointestinal
GLP	Good Laboratory Practice
GRAS	generally recognised as safe
GRN	GRAS notification
HACCP	Hazard Analysis Critical Control Points
HPLC-RI	high performance liquid chromatography refractive index
HPLC-UV	high performance liquid chromatography with ultraviolet detection
ICR	Institute for Cancer Research
ISO	International Organization for Standardization
ISO/IEC	International Organization for Standardization/International Electrotechnical Commission
IUPAC	International Union of Pure and Applied Chemistry
JMHLW	Japanese Ministry of Health Labor and Welfare
JMOH	Ministry of Health and Welfare Government of Japan
LC-MS	liquid chromatography - mass spectrometry
LOAEL	lowest observed adverse effect level
LOD	limit of detection
LOQ	limit of quantification
MFDS	Ministry of Food and Drug Safety in Korea
MGH	monoglucosyl hesperidin
MN	micronuclei
NA	not available
ND	not detected
NDA	EFSA Panel on Nutrition Novel Foods and Food Allergens
NF	novel food
NMR	nuclear magnetic resonance
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Co-operation and Development
P5	5th percentile
P95	95th percentile
RH	relative humidity
RPD	relative population doubling
SC	Scientific Committee
SD	Sprague Dawley
SPF	specific pathogen-free
TAMC	total aerobic microbial count
TG	test guideline
TK	thymidine kinase
T_{max}	time required to reach the maximum concentration
USP	United States Pharmacopeia
w/v	weight per volume
WIST	Wistar

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CONFLICT OF INTEREST

If you wish to access the declaration of interests of any expert contributing to an EFSA scientific assessment, please contact interestmanagement@efsa.europa.eu.

REQUESTOR

European Commission

QUESTION NUMBER

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MAP DISCLAIMER

The designations employed and the presentation of material on any maps included in this scientific output do not imply the expression of any opinion whatsoever on the part of the European Food Safety Authority concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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APPENDIX A

Summary of the 90-day repeated dose oral toxicity study

Study title	90-day repeated dose oral toxicity study (Unpublished study report, 2005b)
Tested system *(No/group)*	Species/strain: Rat, HanRcc:WIST (SPF) *(10/sex/group)*
Test material	The novel food (78.1% as monoglucosyl-hesperidin, 16% as hesperidin) Note: The certificate of analysis was reissued by the applicant in 2024 based on the raw data, including the production and analysis dated of the productions lots used
Dose/concentration	0, 4500, 15,000 and 50,000 ppm in feed corresponding to 0, 279, 927, 3084 mg/kg bw/day for males and 0, 322, 1064, and 3428 mg/kg bw for females, based on food consumption
Method	OECD 408 (1998)

Key results

		Dose groups (ppm in diet)			
Parameter	**Sex**	**0 (control, G1); mean ± (SD)**	**4500 (low, G2); mean ± SD**	**15,000 (intermediate, G3); mean ± SD**	**50,000 (high, G4); mean ± SD**
Haematology					
Reticulocytes [G/L] *Historical control range (P5–P95):* *M: 132–245* *F: 129–259*	M	159 ± 20	162 ± 19	166 ± 15	186** ± 19
	F	185 ± 21	173 ± 24	216 ± 46	184 ± 32
Lymphocytes [G/L] *Historical control range (P5 – P95):* *M: 3.10–6.65* *F: 1.68–4.44*	M	4.78 ± 0.81	4.76 ± 0.81	4.54 ± 0.71	5.79 ± 1.45
	F	2.62 ± 0.43	3.13 ± 0.61	3.13 ± 1.33	3.75* ± 0.87
Large unstained cells, [G/L] *Historical control range (P5 – P95):* *M: 0.02–0.11* *F: 0.01–0.06*	M	0.06 ± 0.03	0.05 ± 0.02	0.07 ± 0.03	0.08 ± 0.05
	F	0.03 ± 0.02	0.03 ± 0.01	0.03 ± 0.02	0.05* ± 0.02
White blood cells [G/L] *Historical control range (P5 – P95):* *M: 4.26–8.53* *F: 2.32–5.74*	M	6.84 ± 1.13	6.70 ± 0.81	6.38 ± 1.07	7.85 ± 1.74
	F	3.50 ± 0.62	4.02 ± 0.66	4.12 ± 1.65	4.73* ± 0.94
Clinical Chemistry					
Sodium [mmol/L] *Historical control range (P5 – P95):* *M: 138.3–147.4* *F: 137.8–147.0*	M	144.1 ± 0.9	144.5 ± 0.6	145.9* ± 0.7	146.2* ± 1.6
	F	144.2 ± 1.1	144.5 ± 0.9	143.8 ± 0.9	144.9 ± 0.7
Chloride [mmol/L] *Historical control range (P5 – P95):* *M: 99.6–108.4* *F: 101.3–109.7*	M	104.7 ± 1.0	105.6 ± 0.9	106.1** ± 0.7	105.5 ± 0.8
	F	106.6 ± 1.6	106.7 ± 1.4	106.6 ± 0.9	107.5 ± 1.6

(Continues)

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Parameter	Sex	Dose groups (ppm in diet) 0 (control, G1); mean ± (SD)	4500 (low, G2); mean ± SD	15,000 (intermediate, G3); mean ± SD	50,000 (high, G4); mean ± SD
Phosphorus [mmol/L] *Historical control range (P5 – P95):* *M: 1.43–2.14* *F: 0.97–1.83*	M	1.80 ± 1.16	1.74 ± 0.09	1.82 ± 0.18	1.96* ± 0.15
	F	1.31 ± 0.08	1.43 ± 0.18	1.43 ± 0.20	1.54* ± 0.16
Organ weights					
Testes	M	4.18 ± 0.34	3.80 ± 0.20*(−9.1%)	3.92 ± 0.28	3.80 ± 0.36* (−9.1%)
Testes/Brain weight ratio	M	204.04 ± 16.25	184.03 ± 21.47**	192.21 ± 13.08	186.20 ± 15.73*
Body weight					
Day 8 (week 2)	M	237.8 ± 7.2	234.1 ± 6.1	230.7 ± 10.1	227.8 ± 10.2*

* $p < 0.05$.

** $p < 0.01$.

APPENDIX B

Overview of human studies

Reference	Study design	Subject characteristics at baseline	Duration of study	Intervention	Endpoints assessed	Safety-related findings
Yuasa et al. (2005)	Study 1: Placebo-controlled, double-blind, parallel group Study 2: Uncontrolled, open-label study Study 3: Uncontrolled, open-label study Study 4: Uncontrolled, open-label study Efficacy and safety (dose-range finding) study on the effects of tea beverages containing MGH on blood lipids	Study 1: Adults (20–65 years old) ($n = 51$; 36 males and 15 females) with serum TG levels 120–300 mg/dL; Study 2: Adults ($n = 10$; 4 males and 6 females) with serum TG levels 30–120 mg/dL; Study 3: Adults ($n = 13$; 9 males and 4 females) with serum TG levels 120–300 mg/dL; Study 4: Adults ($n = 9$; 5 males and 4 females) with serum TG levels 30–120 mg/dL	Study 1: 12 weeks; Study 2: 12 weeks; Study 3: 4 weeks; Study 4: 4 weeks	Study 1: 0 (control beverage) or 340 mg MGH/day in tea beverage Study 2: 340 mg MGH/day in tea beverage Study 3: 1020 mg MGH/day in tea beverage Study 4: 1020 mg MGH/day in tea beverage (MGH not provided by the applicant)	Serum TG, TC, LDL-C, HDL-C, VLDL-C, RLP-C, small particle LDL ratio, mean LDL particle size, phospholipid, apo A-I, A-II, B, C-II and C-II and E, free fatty acid, total ketone body, urinalysis, blood pressure and pulse rate, haematology (WBC, RBC, haemoglobin, haematocrit, platelet count) and clinical chemistry	Study 1, 2, 3 & 4: no changes in the endpoints assessed which could be considered as adverse
Kozuma et al. (2007)	Randomised, double-blind, placebo-controlled, parallel study Efficacy and safety study on the effect of GH on blood pressure when consumed in combination with low-sodium (8.5%) soy sauce-type seasoning	Hypertensive adults (30–65 years old) ($n = 179$, 167 completed the safety evaluation) with SBP 130–159 mmHg and DBP 85–99 mmHg)	12 weeks + 4 weeks of follow-up	Regular soy sauce (control) or 35 mg MGH/15 mL of soy sauce per day (MGH provided by the applicant)	Blood pressure and pulse rate, haematology (exact parameters not reported), clinical chemistry and urinalysis, adverse events	No changes in the endpoints assessed which could be considered as adverse
Kometani et al. (2008)	Randomised, double-blind, placebo-controlled parallel study Efficacy study on the GH effect on the treatment of arthritis	Adults ($n = 19$) with RA and receiving standard antirheumatic therapy	12 weeks	Placebo beverage or 3 g GH/100 mL of beverage per day (unknown supplier)	Standard haematology (WBC, RBC, platelets, haematocrit, haemoglobin), clinical chemistry and urinalysis, adverse events, ACR criteria for diagnosis of RA	No changes in the endpoints assessed which could be considered as adverse. None of the adverse events was related with the consumption of test substance Note: *This study also included investigation of absorption of GH with another group of subjects ($n = 7$). Plasma samples were analysed for metabolite hesperetin, as the amount of absorbed GH. $T_{max} = 6h$. $AUC_{0\text{-}24\,h}$ was ~ 3× higher than for hesperidin*

(Continues)

(Continued)

Reference	Study design	Subject characteristics at baseline	Duration of study	Intervention	Endpoints assessed	Safety-related findings
Nakagawa et al. (2008)	Study 1: Randomised, double-blind, placebo-controlled, parallel study; Study 2: Uncontrolled, open-label; Efficacy study on the effect of GH on blood lipids (study 1) and safety (study 2)	Study 1: Adults (20–65 years old) ($n = 88$, 85 completed the study) with serum TG levels 120–200 mg/dL, taking no lipid-lowering medication; Study 2: Adults (20–65 years old) ($n = 30$, 28 completed the study) with normal TG levels	Study 1: 12 weeks; Study 2: 4 weeks	Study 1: Green tea (control) or 340 mg GH/130 mL of green tea infusion per day; Study 2: 1020 mg GH/day (GH provided by the applicant)	Blood tests: haematological parameters (WBC, RBC, haemoglobin, haematocrit and platelet count), clinical chemistry, urinalysis, physical examination (health conditions, adverse events)	Study 1 & 2: No changes in the endpoints assessed which could be considered as adverse. None of the adverse events was related with the consumption of test substance
Hanawa, Morimoto, Yokomizo, Akaogi, Mafune, Tsunoda, Azuma, Nishitani, Kajimoto, and Kadowaki (2008)	Randomised, double-blind, placebo-controlled, parallel-study Efficacy study to investigate the effect of GH on body weight and body fat	Adults (30–60 years old) ($n = 119$, 83 males and 36 females, 115 completed the study) with BMI 23–30 kg/m^2	12 weeks	Placebo or 250 mg GH/day, in tablets (not provided by the applicant)	Evaluation of abdominal fat area, physical examination, blood pressure and pulse rate, blood tests: lipid profile, haematological (WBC, RBC, haemoglobin, haematocrit, MCV, MCH, MCHC, platelets) and clinical chemistry, urinalysis, adverse events	No changes in the endpoints assessed which could be considered as adverse Several adverse events could not have been ruled out as not related to the test substance: abnormal hepatic function tests (1 case), systemic nonspecific skin rash/urticaria (1 case) and mild symptoms of hypogeusia/taste disorder (1 case)
Hanawa, Morimoto, Yokomizo, Akaogi, Mafune, Tsunoda, Azuma, Nishitani, and Kajimoto (2008)	Randomised, double-blind, placebo-controlled, parallel study Dose-range finding study to evaluate the effect of GH on body weight and serum TG levels	Adults ($n = 93$, 70 males and 23 females) with BMI 19–30 kg/m^2 and serum TG ≥ 120 mg/dL)	4 weeks	0, 250, 500 and 1000 mg GH/day (not provided by the applicant, and no further information on the food matrix)	Physical examination, blood pressure and pulse rate, blood tests: lipid profile, haematological pararmeters (WBC, RBC, haemoglobin, haematocrit, MCV, MCH, MCHC, platelet count) and clinical chemistry, adverse events	No changes in the endpoints assessed which could be considered as adverse Adverse event with unclear relationship with the test substance was a transient right-side abdominal pain (in low-dose group, 1 case). Other adverse events were considered as not related to the test substance
Tanaka et al. (2010)	Study 1: Randomised, double-blind, placebo-controlled study Study 2: Uncontrolled single-arm trial Efficacy (study 1) and safety (study 2) study to investigate the effect of MGH on serum TG levels	Study 1: Adults ($n = 112$, 47 males and 52 females, 99 completed the study) with serum TG levels 120–200 mg/dL; Study 2: Healthy adults ($n = 44$, 17 males and 17 females, 34 completed the study)	Study 1: 12 weeks; Study 2: 4 weeks	Study 1: Placebo beverage or 340 mg MGH/500 mL of beverage per day Study 2: 1030 mg MGH /500 mL of beverage per day (unknown supplier)	Physical examination, blood pressure and pulse rate, blood tests: lipid profile, haematological parameters (WBC, RBC, haemoglobin, haematocrit, lymphocytes, monocytes, neutrophils, MCHC, MCH, MCV, eosinophils, basophils, platelet count) and clinical chemistry, urinalysis, adverse events	Study 1 & 2: No changes in the endpoints assessed which could be considered as adverse None of the adverse events was related with the consumption of the test substance

(Continued)

Reference	Study design	Subject characteristics at baseline	Duration of study	Intervention	Endpoints assessed	Safety-related findings
Ohara et al. (2016)	Randomised, double-blind, placebo-controlled study Efficacy study on the effect of GH in combination with caffeine on body fat and serum TG levels	Adults (20–65 years old) ($n = 160$, 38 males and 37 females, 75 completed the study) with BMI 24–30 kg/m^2 and serum TG levels 100–250 mg/dL	12-week	Five groups: 0 or 470[a] mg GH/day in tablets with 0, 25, 50 or 75 mg of caffeine (GH was provided by the applicant)	Evaluation of abdominal fat area, blood lipid profile, haematological parameters (WBC, RBC, haemoglobin, haematocrit, platelets, MCV, MCH, MCHC) and clinical chemistry, urinalysis, abdominal fat area, adverse events	No changes in the endpoints assessed which could be considered as adverse None of the adverse events was related with the consumption of the test substance

Abbreviations: γ-GT, γ-glutamyl-transferase; ACR, American College of Rheumatology; ALT, alanine aminotransaminase; apo, apolipoprotein; AST, aspartate aminotransaminase; AUC, area under the curve; BMI, body-mass index; DBP, diastolic blood pressure; F, female; GOT, glutamic-oxalacetic transaminase; GPT, glutamic-pyruvic transaminase; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; M, male; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume; MGH, monoglucosyl-hesperidin; RA, rheumatoid arthritis; RBC, red blood cells; RLP-C, remnant-like particle cholesterol; SBP, systolic blood pressure; SD, standard deviation; TC, total cholesterol; TG, triglyceride; VLDL-C, very low density lipoprotein; WBC, white blood cells.

[a]As reported in erratum: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5427583/.

ANNEX A

Dietary exposure estimates to the Novel Food for each population group from each EU dietary survey

Information provided in this Annex is shown in an Excel file (downloadable at https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2024.8911#support-information-section).