SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 27 October 2021

doi: 10.2903/j.efsa.2021.6938

Safety of *Wolffia globosa* powder as a Novel food pursuant to Regulation (EU) 2015/2283

EFSA Panel on Nutrition, Novel Foods, Food Allergens (NDA), Dominique Turck, Torsten Bohn, Jacqueline Castenmiller, Stefaan De Henauw, Karen Ildico Hirsch-Ernst, Alexandre Maciuk, Inge Mangelsdorf, Harry J McArdle, Androniki Naska, Carmen Pelaez, Kristina Pentieva, Alfonso Siani, Frank Thies, Sophia Tsabouri, Marco Vinceti, Francesco Cubadda, Thomas Frenzel, Marina Heinonen, Miguel Prieto Maradona, Rosangela Marchelli, Monika Neuhäuser-Berthold, Morten Poulsen, Josef Rudolf Schlatter, Henk van Loveren, Eirini Kouloura and Helle Katrine Knutsen

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 *Wolffia globosa* powder as a novel food (NF) pursuant to Regulation (EU) 2015/2283. *Wolffia globosa* is an aquatic plant, one out of the 38 species of the water lentil family which is composed by five genera (i.e. *Spirodela*, *Landoltia*, *Lemna*, *Wolffiella* and *Wolffia*). The NF is produced by cultivation of *Wolffia globosa* plants under controlled conditions, washing with hot water and drying. The main constituents of the NF are protein, fibre and fat. The Panel notes that the concentration of trace elements and contaminants in the NF is highly dependent on the conditions of cultivation of the plant and the fertiliser composition. The NF is intended to be used as food ingredient in a variety of food categories and as food supplement. The target population is the general population except for food supplements which are intended to be consumed exclusively by adults. The Panel considers that with the exception of concerns related to the manganese intake, taking into account the composition of the NF and the proposed conditions of use, the consumption of the NF is not nutritionally disadvantageous. The subchronic toxicity study provided with the NF revealed a number of significant findings and the Panel considers the middle dose (6.5 g/kg body weight (bw) per day) as the no observed adverse effect level (NOAEL). Based on the protein concentration, the Panel considers that the consumption of the NF may trigger allergic reactions. The Panel concluded that an increase in manganese intake from the NF used as food ingredient or food supplements is of safety concern and the safety of the NF cannot be established.

Keywords: Novel food, food supplement, isolated from plants, water lentil, duckweed, *Wolffia globosa*

Requestor: European Commission

Question number: EFSA-Q-2019-00695

Correspondence: nda@efsa.europa.eu

Panel members: Dominique Turck, Torsten Bohn, Jacqueline Castenmiller, Stefaan De Henauw, Karen Ildico Hirsch-Ernst, Helle Katrine Knutsen, Alexandre Maciuk, Inge Mangelsdorf, Harry J McArdle, Androniki Naska, Carmen Pelaez, Kristina Pentieva, Alfonso Siani, Frank Thies, Sophia Tsabouri and Marco Vinceti.

Declarations of interest: The declarations of interest of all scientific experts active in EFSA's work are available at https://ess.efsa.europa.eu/doi/doiweb/doisearch.

Suggested citation: EFSA NDA Panel (EFSA Panel on Nutrition, Novel Foods, Food Allergens), Turck D, Bohn T, Castenmiller J, De Henauw S, Hirsch-Ernst KI, Maciuk A, Mangelsdorf I, McArdle HJ, Naska A, Pelaez C, Pentieva K, Siani A, Thies F, Tsabouri S, Vinceti M, Cubadda F, Frenzel T, Heinonen M, Prieto Maradona M, Marchelli R, Neuhäuser-Berthold M, Poulsen M, Schlatter JR, van Loveren H, Kouloura E and Knutsen HK, 2021. Scientific Opinion on the safety of *Wolffia globosa* powder as a Novel food pursuant to Regulation (EU) 2015/2283. EFSA Journal 2021;19(12):6938, 25 pp. https://doi.org/10.2903/j.efsa.2021.6938

ISSN: 1831-4732

The EFSA Journal is a publication of the European Food Safety Authority, a European agency funded by the European Union.

Table of contents

1. Introduction

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

On 24 September 2019, the company 'Hinoman' submitted a request to the European Commission in accordance with Article 10 of Regulation (EU) No 2015/2283[1] to authorise the placing on the market of *Wolffia globosa* powder as a novel food (NF).

The applicant requested to authorise the use of *Wolffia globosa* powder as ingredient in various food categories including food supplements. The target population is the general population. The applicant has requested data protection according to the provisions of Article 26 of Regulation (EU) 2015/2283.

On 25 May 2020, 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 the safety of *Wolffia globosa* powder as a novel food.

1.2. Additional information

The NDA Panel assessed previously the safety of water lentil powder from Lemnaceae as Novel Food intended for consumption as food ingredient and food supplement (EFSA NDA Panel, 2021). The novel food consisted of species from the *Lemna* genus (70–100%) and the *Wolffia* genus (0–30%). In its Scientific opinion, the Panel concluded that the increase in manganese intake from the NF was substantial as compared to the background manganese dietary intake, and consequently, the safety of the NF could not be established.

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 requests for supplementary information. During the assessment, the Panel identified additional data which were not included in the application.

Administrative and scientific requirements for NF applications referred to in Article 10 of Regulation (EU) 2015/2283 are listed in the 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: identification of plant material letter (Appenroth, 2014), *in vivo* determination of PDCAAS (Product Safety Labs, 2015), endophytic bacteria screening analyses, GRAS notification report (Ramboll Environ, 2015), bacterial reverse mutation test (Kawamata et al., 2020), *in vitro* mammalian cell micronucleus test (Kawamata et al., 2020), repeated dose 4-day oral toxicity study (Pharmaseed Ltd, 2016-unpublished), two repeated dose 28-day oral toxicity studies (APS, 2018a-unpublished and APS, 2018b-unpublished), iron supplementation study in rats (Yaskolka Meir et al., 2019), 90-day repeated dose oral toxicity study (non-GLP) (NPHI, 2018-unpublished), 90-day repeated dose oral toxicity study (Kawamata et al., 2020).

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 the Commission Implementing Regulation (EU) 2017/2469.

[1] Regulation (EU) 2015/2283 of the European Parliament and of the Council of 25 November 2015 on novel foods, amending Regulation (EU) No 1169/2011 of the European Parliament and of the Council and repealing Regulation (EC) No 258/97 of the European Parliament and of the Council and Commission Regulation (EC) No 1852/2001. OJ L 327, 11.12.2015, p. 1–22.

[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.

Additional information which was not included in the application was retrieved by literature search following a search strategy and standard operating procedure as described by UCT Prague (2020).

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 which is the subject of the application is *Wolffia globosa* powder, produced by cultivation and drying of the aquatic plant *Wolffia globosa*. The NF is proposed to be used as food ingredient in a variety of food categories and as food supplement. The target population is the general population.

The applicant indicated that in accordance with Article 3 of the NF Regulation (EU) 2015/2283, the NF falls under the category 'food consisting of, isolated from, or produced from plants and their parts' except when the food has a history of safe food use within the Union and is consisting of, isolated from or produced from a plant or a variety of the same species obtained by:

- traditional propagating practices which have been used for food production within the Union before 15 May 1997; or
- non-traditional propagating practices which have not been used for food production within the Union before 15 May 1997, where those practices do not give rise to significant changes in the composition or structure of the food affecting its nutritional value, metabolism or level of undesirable substances.

3.2. Identity of the NF

The NF is a powder produced from the aquatic plant *Wolffia globosa* (Roxb.) Hartog & Plas. *Wolffia globosa* is one out of the 38 species that comprise the water lentil family represented by species of five genera (i.e. *Spirodela*, *Landoltia*, *Lemna*, *Wolffiella* and *Wolffia*) and belonging to the Araceae family and the Lemnoideae subfamily (Cabrera et al., 2008; Wang et al., 2010). Water lentils are flowering aquatic plants which float or are submersed in water and consist of green bodies called fronds. Reproduction of water lentils is achieved mostly by asexual budding-vegetative reproduction whereas sexual reproduction via flowering occasionally occurs. *Wolffia globosa* is composed of ovoid fronds of 0.4–0.8 mm. Budding of *Wolffia globosa* is observed via funnel-shaped pouches at the basal end of the fronds which is a characteristic feature of those plant species and used for their authentication.

The NF is a green powder obtained after drying the fresh plant material. The plant used for the production of the NF was authenticated in 2014 as *Wolffia globosa* by Dr. Klaus J Appenroth (University of Jena, Germany, Head of the International Steering Committee on Duckweed Research and Application) based on morphological and microscopic characteristics (Appenroth, 2014, confidential/data protected). The cultivated strain was patented by the applicant as *Wolffia globosa* Mankai.

3.3. Production process

According to the information provided, the NF is produced in line with Good Manufacturing Practice (GMP) and Hazard Analysis Critical Control Points (HACCP) principles.

The cultivation of *Wolffia globosa* plants is performed in PVC-sealed basins in greenhouses or in a semi-open mesh net building construction under controlled conditions. Water and fertilisers are used for the growth of the plant material while no pesticides or fungicides are added in the growth media. Harvesting is completed semi or fully automatically. The fresh plant material is washed with hot water and subsequently dried using a temperature-controlled dehydrator or a freeze-dryer. The dry product is packed and sealed in bags of laminated aluminium under modified atmosphere. The product is stored at 25°C and humidity < 60%.

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

3.4. Compositional data

The NF is mainly composed of proteins 43–46%, carbohydrates 35–40%, fat 9.5–12%, ash 2.8–8.3% and moisture 2.6–4.4%.

In order to confirm that the manufacturing process is reproducible and adequate to produce on a commercial scale a product with the required characteristics, the applicant provided analytical information for three to seven independently produced batches of the NF, depending on the parameter.

The applicant submitted analytical data for proximate parameters as presented in Table 1.

Table 1: Batch to batch analysis of the NF

Parameters	Unit	Batch							Method of analysis
		#1	#2	#3	#4	#5	#6	#7	
Moisture and volatiles	%	NA	2.6	4.2	4.4	NA	3.2	3.4	AOAC 925.09
Protein (N × 6.25)	%	NA	45.62	43.17	44.92	45.13	45.44	43.31	AOAC 990.03; AOAC 992.15
Crude fat	%	9.47	10.60	NA	NA	NA	NA	11.86	AOAC 2001.11 (by acid hydrolysis)
Total dietary fibre	%	31.40	32.30	35.30	34.20	27.80	28.70	27.70	AOAC 991.43
Fibre (acid detergent)	%	NA	11.1	19.8	25.0	28.2	12.1	NA	Gravimetry (In-house)
Fibre (neutral detergent)	%	NA	12.4	19.7	24.5	25.8	17.6	NA	Gravimetry (In-house)
Ash	%	6.29	7.42	8.15	8.34	2.82	4.11	4.07	AOAC 942.05
Carbohydrates	%	NA	36.02	37.29	35.33	39.91	38.01	40.03	CFR 21-calc.
Haemicellulose	%	NA	1.3	< 1.0	< 1.0	< 1.0	5.5	NA	Gravimetry (In-house)
Lignin	%	NA	< 0.5	0.6	0.9	1.5	1.9	NA	Gravimetry (In-house)
Cellulose	%	NA	0.11	0.19	0.24	0.27	0.10	NA	Gravimetry (In-house)
Starch	%	NA	NA	3.2	3.6	4.2	3.5	NA	AOAC 996.11

AOAC: Association of Official Analytical Collaboration; CFR: Code of Federal Regulations; NA: not available.

In order to characterise further the composition of the NF, the applicant provided analytical data on nutritionally relevant components and other naturally occurring compounds in the NF, namely micronutrients and trace elements, amino acids, carbohydrates, vitamins, fatty acids, catechins, phenolic acids and carotenoids.

The variability of micronutrients and trace elements among seven batches is presented in Table 2.

Table 2: Concentration of micronutrients, trace elements and heavy metals in seven batches of the NF

Parameters	Unit	Batch							Method of analysis
		#1	#2	#3	#4	#5	#6	#7	
Aluminium	mg/kg	3.3	12	4.6	4.2	< 10	< 10	16	AOAC 993.14 Mod.; AOAC 965.17/985.01 mod.; DIN EN ISO 11885, mod., CON-PV 00006, ICP-OES
Barium	mg/kg	4.9	6.8	7.4	5.4	8.2	26.7	5.3	AOAC 993.14 Mod.;J.AOAC 92, 1484-1518 (2009); DIN EN ISO 11885, mod., CON-PV 00006, ICP-OES
Boron	mg/kg	59.7	53.9	36.0	31.0	40.2	45.4	39.8	AOAC 993.14 Mod.;J.AOAC 92, 1484-1518 (2009); DIN EN ISO 11885, mod., CON-PV 00006, ICP-OES
Calcium	mg/kg	5,640	5,130	4,400	4,500	6,880	10,400	5,750	AOAC 965.17/985.01 mod.; AOAC 993.14; AOAC 984.27, 927.02, 985.01, 965.17 mod; DIN EN ISO 11885, mod., CON-PV 00006, ICP-OES

Parameters	Unit	Batch							Method of analysis
		#1	#2	#3	#4	#5	#6	#7	
Chromium	mg/kg	0.6	4.0	3.5	2.3	< 1.2	1.4	13	AOAC 993.14 Mod.;DIN EN ISO 11885, mod., CON-PV 00006, ICP-OES
Cobalt	mg/kg	0.15	< 0.75	< 0.2	< 0.2	< 0.75	< 0.75	< 0.75	AOAC 993.14 Mod.;AOAC 965.17/968.08 modified; DIN EN ISO 11885, mod., CON-PV 00006, ICP-OES
Copper	mg/kg	20.3	17	14.0	18.0	NA	NA	20	AOAC 993.14 Mod.; AOAC 965.17/968.08 modified; DIN EN ISO 11885, mod., CON-PV 00006, ICP-OES
Iron	mg/kg	387	723	600	450	534	1,590	726	AOAC 965.17/985.01 mod.; AOAC 984.27, 927.02; DIN EN ISO 11885, mod., CON-PV 00006, ICP-OES
Lithium	mg/kg	NA	NA	NA	NA	0.5	0.5	< 0.2	J.AOAC 92, 1484-1518 (2009)
Magnesium	mg/kg	1,910	1,950	1,800	1,800	1,680	2,070	1,530	AOAC 993.14 Mod.;DIN EN ISO 11885, mod., CON-PV 00006, ICP-OES
Manganese	mg/kg	226	292	210	200	206	197	219	AOAC 993.14 Mod.; AOAC 965.17/985.01 mod.; DIN EN ISO 11885, mod., CON-PV 00006, ICP-OES
Molybdenum	mg/kg	3.3	3.6	3.5	5.1	< 1.2	< 1.2	< 1.2	AOAC 993.14 Mod.; AOAC 965.17/968.08 modified; DIN EN ISO 11885, mod., CON-PV 00006, ICP-OES
Nickel	mg/kg	0.273	1.3	1.4	1.0	0.23	0.22	5.64	AOAC 993.14 Mod.; AOAC 965.17/968.08 modified; DIN EN ISO 11885, mod., CON-PV 00006, ICP-OES
Potassium	mg/kg	22,700	22,900	29,000	31,000	1,340	1,290	8,720	AOAC 993.14 Mod.; AOAC 965.17/985.01 mod.;AOAC 984.27, 927.02;DIN EN ISO 11885, mod., CON-PV 00006, ICP-OES
Phosphorus	mg/kg	8,440	8,300	9,800	9,800	4,730	6,750	6,240	AOAC 993.14 Mod.; AOAC 965.17/985.01 mod.; DIN EN ISO 11885, mod., CON-PV 00006, ICP-OES
Selenium	mg/kg	< 0.23	< 0.1	< 0.2	< 0.2	< 0.1	< 0.1	< 0.1	AOAC 993.14 Mod.; DIN EN ISO 11885, mod., CON-PV 00006, ICP-MS
Silica (SiO_2)	mg/kg	22	24	NA	NA	< 20	56	110	AOAC 986.15 mod.; DIN EN ISO 11885, mod., CON-PV 00006, ICP-OES
Silicon (Si)	mg/kg	10	11	22.0	33.0	< 10	26	50	
Sodium	mg/kg	309	420	530	650	250	390	330	AOAC 965.17/985.01 mod.; DIN EN ISO 11885, mod., CON-PV 00006, ICP-OES
Strontium	mg/kg	49.3	54.1	50.0	50.0	46.2	122.2	44.7	AOAC 993.14; AOAC 2013.06; DIN EN ISO 11885, mod., CON-PV 00006, ICP-OES

Parameters	Unit	Batch							Method of analysis
		#1	#2	#3	#4	#5	#6	#7	
Tin	mg/kg	0.22	< 25	NA	NA	NA	NA	NA	AOAC 993.14; AOAC 965.17/ 968.08 modified
Titanium	mg/kg	0.83	2.6	0.9	0.6	0.5	< 0.4	1.4	AOAC 993.14 Mod.
Vanadium	mg/kg	0.213	< 0.20	< 0.2	< 0.2	< 0.20	< 0.20	< 0.20	AOAC 993.14 Mod.; DIN EN ISO 11885, mod., CON-PV 00006, ICP-MS
Zinc	mg/kg	363	264	110	190	90	76	217	AOAC 993.14 Mod.; AOAC 965.17/985.01 mod.; DIN EN ISO 11885, mod., CON-PV 00006, ICP-OES
Heavy metals									
Arsenic[(a)]	mg/kg	0.031	0.064	< 0.1	0.3	0.014	0.030	0.018	J. AOAC vol. 90 (2007) 844-856 (Mod); AOAC 993.14 Mod. EN 15763:2009, CON-PV 01274, ICP-MS
Cadmium[(b)]	mg/kg	0.06	0.04	0.02	0.04	< 0.01	< 0.01	< 0.01	J. AOAC vol. 90 (2007) 844-856 (Mod); AOAC 993.14 Mod. EN 15763:2009, CON-PV 01274, ICP-MS
Lead[(c)]	mg/kg	0.23	0.50	0.33	0.92	0.07	0.09	0.05	J. AOAC vol. 90 (2007) 844-856 (Mod); AOAC 993.14 Mod. EN 15763:2009, CON-PV 01274, ICP-MS
Mercury[(d)]	mg/kg	< 0.012	< 0.011	< 0.005	< 0.005	< 0.010	0.014	< 0.010	J. AOAC vol. 90 (2007) 844-856 (Mod); AOAC 993.14 Mod. EN 15763:2009, CON-PV 01274, ICP-MS

AOAC: Association of Official Analytical Collaboration; CON-PV: methods of Eurofins WEJ Contaminants GmbH; DIN: Deutsches Institut für Normung e.V.; EN: Europäische Norm (European Standards); ISO: International Organization for Standardization; ICP-OES: Inductively Coupled Plasma Optical Emission Spectroscopy; ICP-MS: Inductively Coupled Plasma Mass Spectrometry; NA: not available.
(a): Additional analytical data were provided for two batches #8 and #9 (i.e. 0.019 and 0.040 mg/kg).
(b): Additional analytical data were provided for two batches #8 and #9 (i.e. 0.014 and 0.005 mg/kg).
(c): Additional analytical data were provided for two batches #8 and #9 (i.e. 0.112 and 0.222 mg/kg).
(d): Additional analytical data were provided for two batches #8 and #9 (i.e. < 0.005 and < 0.005 mg/kg).

Following a request from EFSA, the applicant modified the fertiliser composition and provided analytical data for trace elements for six additional batches (Table 3).

Table 3: Trace element concentration in six additional batches of the NF

Parameter*	Unit	Batch number					
		#10	#11	#12	#13	#14	#15
Aluminium	mg/kg	< 3	3.6	4.0	< 3	3.6	< 3
Boron	mg/kg	22.5	41.8	56.8	29.8	31.3	21.3
Copper	mg/kg	8.5	6.0	6.3	8.0	8.5	6.3
Iron	mg/kg	570.5	900.5	844.8	348.3	369.0	299.7
Manganese	mg/kg	116.4	59.6	116.3	105.8	101.6	69.5
Molybdenum	mg/kg	< 1	< 1	< 1	< 1	< 1	< 1
Zinc	mg/kg	136.7	158.9	91.6	170.0	165.9	112.3

*: Parameters were analysed by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES).

The applicant investigated the carbohydrate profile in six batches of the NF providing analytical data on the concentration of monosaccharides (i.e. fructose, glucose) and disaccharides (i.e. sucrose, lactose, maltose). All sugars were found below the limit of detection (LOD < 0.15%) measured by high-performance liquid chromatography – refractive index (HPLC-RI).

The concentration of a panel of vitamins was reported in seven batches of the NF (Table 4).

Table 4: (Pro-)Vitamin content of seven batches of the NF

Parameter	Unit	Batch number								Method of analysis
		#1	#2	#3	#4	#5	#6	#16	#7	
Beta-carotene	µg/100 g	46,200	20,100	31,860	28,980	36,000	73,200	61,200	49,800	AOAC 974.29 Mod. Official Methods of Analysis, Methods 992.04, 992.06, and 2001.13, AOAC International (Mod).
Total vitamin A	RAE/100 g	3,850	1,712	2,655	2,553	3,000	6,100	5,100	4,150	
Biotin	mg/100 g	0.0487	0.0564	0.0518	0.0418	0.0419	0.0428	NA	0.0658	
Folate	mg/100 g	1	0.9	1.5	1.5	0.4	0.4	NA	0.4	Met. of Vitamin Assay, Interscience Publ., Ch.12
Niacin	mg/100 g	6.3	5.5	7.8	7.7	0.3	0.4	NA	1.6	AOAC 992.05 Mod.
Thiamin hydrochloride	mg/100 g	0.9	0.6	0.9	0.7	0.0	0.1	NA	0.1	AOAC 944.13 Mod.
Cobalamin	µg/100 g	4.4	2.2	2.8	2.6	0.7	< 0.4	NA	< 0.4	AOAC 942.23 mod.
Riboflavin	mg/100 g	3.4	3.4	3.5	3.3	0.4	< 0.2	NA	< 0.4	AOAC 952.20/ 45.2.02
Pantothenic acid	mg/100 g	0.851	0.281	0.360	0.300	< 0.055	< 0.055	NA	< 0.152	AOAC 970.65 mod.
Ascorbic acid	mg/100 g	6.8	12.6	18.2	38.0	85.2	151.0	NA	272.0	AOAC 945.74 (mod.)
Alpha-tocopherol	mg/100 g	31.37	16.15	22.2	16.8	23.1	34.4	NA	46.2	AOAC 971.30 with HPLC quantification mod.
Beta-tocopherol	mg/100 g	NA	NA	NA	NA	0.359	0.400	NA	0.661	
Gamma-tocopherol	mg/100 g	NA	NA	NA	NA	0.141	0.143	NA	0.394	
Delta-tocopherol	mg/100 g	NA	NA	NA	NA	< 0.100	< 0.100	NA	< 0.100	
Menaquinone 4	mg/100 g	NA	< 0.1	< 0.1	< 0.1	< 0.2	< 0.2	0.0143	NA	USP 31 NF26 or EN14148 mod.
Menaquinone 7	mg/100 g	NA	< 0.1	< 0.1	< 0.1	< 0.2	< 0.2	0.0146	NA	USP 31 NF26 or EN14148 mod.
Phylloquinone	mg/100 g	NA	6.70	2.56	8.37	8.71	11.10	9.58	5.88	EN 14148:2003 Official Methods of Analysis, Methods 992.27, 999.15, AOAC International (Mod).
Pyridoxine	mg/100 g	1.25	1.25	1.27	1.10	0.27	0.35	NA	0.19	JAOAC 88, 30-37

Parameter	Unit	Batch number								Method of analysis
		#1	#2	#3	#4	#5	#6	#16	#7	
Vitamin D2	IU/100 g	NA	NA	NA	NA	< 4.00	< 4.00	NA	NA	EN 12821:2009 USP (modified)
Vitamin D3	IU/100 g	NA	NA	NA	NA	< 4.00	< 4.00	NA	NA	EN 12821:2009 USP (modified)

AOAC: Association of Official Analytical Collaboration; EN: Europäische Norm (European Standards); USP: United States Pharmacopeia; HPLC: high-performance liquid chromatography; NA: not available.

The fatty acid profile of the NF was provided in nine batches (#1-#7; #16; #17). In total, analytical data for 57 free fatty acids were presented, alpha linolenic acid being the most prominent with an average concentration of 3.9%. The NF contains on average 8% fatty acids, including 2% saturated fatty acids, 0.45% monounsaturated (MUFA), 5.45% polyunsaturated (PUFA) and 0.04% trans fatty acids.

Total polyphenol content is reported to range from 382 to 700 mg/100 g (gallic acid equivalents-GAE) and total catechins from 16.5 to 121 mg/100 g in five batches, with individual catechins and phenolic acids identified. Regarding phenolic acids, the applicant provided analytical data on *p*-coumaric acid (< 3.33–7.73 mg/100 g), ferulic acid (< 3.33–3.97 mg/100 g), caffeic acid (44.5–110 mg/100 g) and sinapic acid (< 3.33–11.9 mg/100 g) in five batches, LOQ 3.33 mg/100 g. In addition, concentrations of lutein (0.89–1.8 mg/100 g) and zeaxanthin (0.03–0.16 mg/100 g) were reported in seven batches.

Regarding antinutritional factors, phytic acid was found below the limit of detection (< 0.14%) in six batches of the NF and oxalic acid was measured between 230 and 637 mg/100 g in nine batches.

The applicant provided batch to batch testing for microbiological parameters in five batches (Table 5).

Table 5: Microbiological parameters in five batches of the NF

Parameter	Unit	Batch number					Method of analysis
		#2	#18	#3	#19	#4	
Total plate count	CFU/g	< 10	75	90	40	55	SI 885/3
Bacillus cereus	CFU/g	< 50	< 50	< 50	< 50	< 50	AOAC 980.31; BAM Chapter 14
Staphylococcus aureus	CFU/g	NA	NA	< 50	< 50	< 50	BAM Chapter 12; SI 885/6
Total Coliforms	CFU/g	< 10	< 10	< 10	< 10	< 10	BAM Chapter 4; SI 885/4
Escherichia coli	CFU/g	< 10	< 10	< 10	< 10	< 10	BAM Chapter 4; ISO 16649-2
***E. coli* O157:H7**	per 30 g	NA	ND	ND	ND	ND	ISO 16654
Moulds	CFU/g	25	< 10	< 10	< 10	< 10	BAM Chapter 18
Yeasts	CFU/g	< 10	< 10	< 10	< 10	< 10	SI 885/8
Listeria monocytogenes	per 25 g	ND	ND	ND	ND	ND	AOAC OMA 2004.02
Faecal streptococci	CFU/g	< 10	< 10	< 10	< 10	< 10	SI 885/5
***Salmonella* spp.**	per 25 g	ND	ND	ND	ND	ND	AOAC 2003.09

AOAC: Association of Official Analytical Collaboration; BAM: Bacteriological Analytical Manual; ISO: International Organisation for Standardization; OMA: Official method of analysis; SI: Israel Standards; ND: not detected; CFU: colony forming units; NA: not available.

The applicant provided analytical data on nitrate content in three batches of the NF ranging between 85 and 2,300 mg/kg. Moreover, concentrations of EDTA (< 380 μg/g) and sulfates (0.25%) were reported in two batches of the NF.

Furthermore, the applicant provided analytical data for the concentration of pesticides, mycotoxins and cyanotoxins which were all reported below the quantification limit (LOQ). Regarding cyanotoxins, the concentrations of anatoxin (LOQ, 10 μg/kg), microcystins (LOQ, 25 μg/kg) and nodularin (LOQ, 25 μg/kg) were below the LOQ in three batches of the NF.

Following a request from EFSA, the applicant investigated the presence of endophytic bacteria in water lentils. Endophytic bacteria were identified in ten samples of *Wolffia globosa* by 16S rRNA sequencing. Some of the identified endophytic bacteria have been associated with the production of indole alkaloids (Gilbert et al., 2018). Thus, the applicant provided analytical data on the concentration of

indole acetic acid (IAA) and indole lactic acid (ILA) in five batches of the NF with moisture content ranging between 2.45% and 4.02% and which were found below the LOQ of the method (0.05 mg/kg).

The laboratories that conducted the analyses presented in the application are accredited in accordance with the recognised International Standard ISO/IEC 17025:2005, ISO/IEC 17011:2017 and ISO/IEC 17025:2018.

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

3.4.1. Stability

The applicant performed stability tests with two independently produced batches of the NF. The tests were carried out under normal storage conditions in nitrogen-sealed packs at room temperature (~ 25°C) and at 60–75% RH (relative humidity) for a period of 18 and 23 months. The batches were analysed for physico-chemical and microbiological parameters. Protein content, water activity, pH and microbiological parameters remained stable during the period of testing. Following a request from EFSA, the applicant provided data on the oxidative stability of the NF. In particular, the applicant measured propanal and hexanal concentration in batches from 0 up to 18 months of age (propanal 0 months: 4.03 mg/kg and 18 months: 9.41 mg/kg; hexanal 0 months: 1.55 mg/kg and 18 months: 6.07 mg/kg).

Based on the provided data, the NF is expected to be stable for 16 months from manufacturing date, under recommended storage conditions in nitrogen-sealed packs at room temperature and humidity < 60%.

In addition, the stability of the NF was tested in yogurt and bread. The testing conditions were not appropriate to draw conclusion on the NF stability in food matrices.

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 6.

Table 6: Specifications of the NF

Description: *Wolffia globosa* powder is dry green free-flowing powder produced from dried *Wolffia globosa* plants	
Source: *Wolffia globosa*	
Parameter	Specification
Moisture	< 5%
Protein (N × 6.25)	40–50%
Ash	2.5–8.5%
Fat	7–10%
Total fibre	27–35%
Beta-carotene	200–750 mg/kg
Phylloquinone	20–120 mg/kg
Folic acid	3–15 mg/kg
Copper	< 8.6 mg/kg
Zinc	< 200 mg/kg
Iron	< 1,000 mg/kg
Manganese	< 116.5 mg/kg
Molybdenum	< 6 mg/kg
Boron	< 60 mg/kg
Oxalic acid	< 6,500 mg/kg

Contaminants	
Nitrate	< 3,100 mg/kg
Arsenic	< 1 mg/kg
Lead	< 1.5 mg/kg
Cadmium	< 0.6 mg/kg
Mercury	< 0.1 mg/kg
Total aflatoxins	< 10 µg/kg
Microcystins	< 25 µg/kg
Nodularins	< 25 µg/kg
Microbiological parameters	
Total plate count	< 10^3 CFU/g
E. coli	ND in 1 g
Coliforms	< 10^2 CFU/g
Moulds	< 10^2 CFU/g
Yeasts	< 10^2 CFU/g
Salmonella spp.	ND in 25 g
S. aureus	ND in 1 g
Listeria monocytogenes	ND in 25 g

CFU: colony forming units; ND: not detected.

The Panel considers that the information provided on the specifications of the NF is sufficient (but see Section 3.9 Nutritional information).

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

3.6.1. History of use of the source

Wolffia arrhiza has been used as a vegetable for many generations in Myanmar, Laos and northern Thailand (Bhanthumnavin and McGarry, 1971). It is harvested twice a week during 9 months of the year (November–July). Its Thai local name 'Khai-nam' means eggs of the water. According to Appenroth et al. (2017), the plant that was widely consumed as food in Southeast Asia was *W. globosa* rather than *W. arrhiza* as mentioned by Bhanthumnavin and McGarry (1971). The applicant noted that *W. globosa* appears in recent articles (Siripahanakul et al., 2013; Shirai and Rambo, 2014) among edible species sold in the market. *Wolffia globosa* appears in several recognised databases as edible species. In particular, the Japan International Research Centre for Agricultural Sciences lists *W. globosa* among local vegetables of Thailand (JIRCAS, 2010). *Wolffia globosa* is also listed in the GRIN database as human and animal food.

Recently, a traditional food notification submitted to the European Commission pursuant to Article 14 of Regulation (EU) 2015/2283 regarding the consumption of *Wolffia globosa* and *Wolffia arrhiza* as fresh vegetable was assessed by EFSA and no duly reasoned safety objections were raised (EFSA, 2021).

3.6.2. History of use of the NF

The NF is on the market in the US (since March 2019) and Israel (since March 2020, after approval in 2019) as food ingredient for several food categories such as plant-based meat alternatives, shakes and baked goods in the form of frozen cubes.

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. In the case of food supplements, the target population is restricted to adults.

3.7.2. Proposed uses and use levels

The NF is proposed to be used as an ingredient in several food products. These food products and the maximum use levels are reported in Table 7. In addition, the applicant intends to market the NF for use in food supplements, at a maximum dose of 20 g per day.

Table 7: Food categories and maximum use levels intended by the applicant

FAIM food category	Food category	Max use level (mg NF/kg)
01.2	Unflavoured milk products	10,000
03	Edible ices	10,000
04.2	Processed vegetables	15,000
06.4	Pasta	20,000
06.5	Noodles	20,000
07.1	Bread and rolls	10,000
14.1.2.2	Vegetable juices	20,000
14.1.3	Fruit nectars as defined by Directive 2001/112/EC and vegetable nectars and similar products	10,000
16	Desserts excluding products covered in category 1, 3 and 4	10,000

FAIM: Food Additives Intake Model.

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 7), using the EFSA FAIM tool which is a tool for estimating chronic dietary exposure to food additives.[3] The FAIM tool is based on individual data from the EFSA Comprehensive European Food Consumption Database (EFSA, 2011). The lowest and highest mean and 95th percentile anticipated daily intake of the NF (on a mg/kg bw basis), among the EU dietary surveys, are presented in Table 8. The highest intake was estimated for infants (age < 1 year) at 730 mg/kg bw per day, at the 95th percentile.

Table 8: Intake estimate resulting from the use of the NF as an ingredient in the intended food categories at the maximum proposed use levels calculated by the FAIM tool

Population group	Age (years)	Mean intake (mg/kg bw per day)		P95th intake (mg/kg bw per day)	
		Lowest[(a)]	Highest[(a)]	Lowest[(b)]	Highest[(b)]
Infants	< 1	43.7	211	170	730
Young children[(c)]	1 to < 3	123	261	241	555
Other children	3 to < 10	79.4	191	155	375
Adolescents	10 to < 18	40.5	95.7	81.1	198
Adults[(d)]	≥ 18	43.2	71.3	75.7	139

bw: body weight; FAIM: Food Additives Intake Model; NF: novel food.
(a): FAIM tool exposure estimate was generated on 01/06/2021. The lowest and the highest averages observed among all EU surveys are reported in these columns.
(b): FAIM tool exposure estimate was generated on 01/06/2021. The lowest and the highest P95th observed among all EU surveys are reported in these columns (P95th based on < 60 individuals are not considered).
(c): Referred as 'toddlers' in the EFSA food consumption comprehensive database (EFSA, 2011).
(d): Includes elderly, very elderly, pregnant and lactating women.

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).

For the intake of the NF in the form of food supplements, the target population is limited to adults and the proposed maximum daily dose of the NF is 20 g per day. Taking into consideration the default body weight of 70 kg for adults (EFSA Scientific Committee, 2012), this corresponds to an intake of 286 mg/kg bw per day.

[3] https://www.efsa.europa.eu/it/applications/food-improvement-agents/tools

3.7.4. Combined intake from the NF and other sources

The NF is intended for use as food ingredient and food supplement. Food supplements are intended only for adult population and the applicant indicated that food supplements containing the NF are not intended to be consumed in combination with foods fortified with the NF. However, the Panel notes that combined intake of the NF from food supplements and foods containing the NF can be expected. The combined intake of the NF from food supplements and foods containing the NF for adults can reach 425 mg/kg bw per day or 29.8 g per day, using 70 kg as the default body weight for adults (EFSA Scientific Committee, 2012).

3.7.5. Estimate of exposure to undesirable substances

EFSA calculated the intake of heavy metals and trace elements from the NF which are highly dependent on the cultivation conditions considering the specification levels for each element (Table 6) and estimated daily intake of the NF for all population groups (Table 8). The Panel considers that exposure to heavy metals and trace elements from the NF is not expected to exceed established maximum levels[4] and upper levels for any population group (SCF/NDA, 2006). The assessment of the intake of manganese (Mn) from the NF, for which upper levels are not available, is provided in Section 3.9.

As accumulation of cyanobacteria toxins is reported in the literature (Mitrovic et al., 2005; Saqrane et al., 2007), exposure to microcystins from the NF was calculated using specifications for microcystins (< 0.025 mg/kg, Table 6) and estimated daily intake of the NF. The highest exposure to microcystins from the NF was calculated for infants, resulting in 0.018 μg/kg bw per day, which is below the tolerable daily intake (TDI) of 0.04 μg/kg bw per day (WHO, 2020).

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

The NF is composed of 40–50% protein, fibre/carbohydrates (27–35%) and fat (7–10%) which are normal constituents of the human diet. The Panel considers the constituents of the NF are expected to undergo normal metabolic processes.

3.9. Nutritional information

The applicant provided a nutritional analysis of the NF.

The applicant presented scoring pattern for indispensable amino acids of the NF for one batch (Batch #20, Table 9). The scoring of the majority of amino acids in this batch was lower as compared to the other seven batches presented in Appendix A. The scoring pattern for this batch, which was used for the *in vivo* determination of the Protein Digestibility Corrected Amino Acid Score (PDCAAS), was compared to indispensable amino acid reference profiles (WHO, 2007) and commonly consumed foods with high protein content (Gorissen et al., 2018).

Table 9: Indispensable amino acid scoring

Parameter (g/100 g protein)	#20	Oat	Pea	Egg	Scoring pattern (indispensable amino acid reference profiles) for adults (WHO, 2007)
Histidine	2.0	1.4	2.0	1.8	1.5
iso-Leucine	3.8	2.0	2.9	3.1	3.0
Leucine	7.2	5.9	7.1	7.1	5.9
Lysine	6.0	2.0	5.9	5.3	4.5
Tyrosine + phenylalanine	8.1	6.6	7.9	8.0	3.8
Threonine	4.0	2.3	3.1	3.9	2.3
Valine	4.9	3.1	3.4	3.9	3.9
Cysteine + methionine	2.8	0.8	0.6	3.5	2.2
Tryptophan	2.1				0.6

[4] Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs (OJ L 364, 20.12.2006, p. 5).

The protein quality of the NF was determined *in vivo* by PDCAAS. In total, 12 male rats were divided into three groups (protein free control/casein control/NF). The protein diets (casein & NF) were formulated to contain 10% protein and animals were administered 15 g/day of their respective diets for 9 days. Faeces samples were collected daily at 24-h interval for the last 5 days of the study for each animal and combined to calculate nitrogen content. The PDCAAS for the NF was 89% similar to soy protein isolate (90%) as reported by Darragh & Hodgkinson (2000).

The applicant provided a randomised study in 36 healthy men where the bioavailability of indispensable amino acids (IAAs) from the NF was assessed by comparison to those from animal and plant protein sources. The subjects consumed three iso-protein (30 g) based test-meals (soft cheese, green peas, NF) after a controlled diet for the three previous days and subsequent overnight (12 h) fast. Blood samples were collected at 0, 30, 90 and 180 min. The trajectory of the concentration of IAAs in blood over 180 min showed significant increases in histidine, phenylalanine, threonine and tryptophan, after consumption of the NF when compared to the baseline, similar to the other protein sources. Leucine/isoleucine and valine also presented a significant increase after consumption of the NF; however, this increase was more prominent upon soft cheese consumption. Lysine and methionine remained at the same levels over the course of the study (Kaplan et al., 2019).

The bioavailability of iron from the NF was investigated in 294 abdominally obese/dyslipidaemic non-anaemic subjects. The participants were divided into three groups (physical activity (PA) and usual diet; physical activity and Mediterranean diet PA + MED; or physical activity, NF and Mediterranean diet PA + green-MED) and a 6-month intervention was followed. Haemoglobin increased in the PA + green-MED group (0.23 g/dL) as compared to PA (−0.1 g/dL; $p < 0.001$) and PA + MED (−0.1 g/dL; $p < 0.001$). Serum-iron and serum transferrin saturation increased in the PA + green-MED group as compared to the PA-group (8.21 μg/dL vs. −5.23 μg/dL and 2.39% vs. −1.15%; $p < 0.05$ for both comparisons), as did folic acid ($p = 0.011$) (Yaskolka Meir et al., 2019).

Iron homoeostasis parameters were also investigated in a complementary animal study. Iron-deficient anaemia-induced female rats ($n = 50$) were divided into six groups, including control and groups treated with ferrous-gluconate (FG) preparations or NF preparations over 22 days. Increase of haemoglobin levels in both FG and NF iso-iron treatments suggests that iron derived from the NF is bioavailable.

The NF contains on average 8% of total fatty acids from which 2% are saturated fatty acids, predominantly palmitic acid (1.7%), 0.45% monounsaturated (MUFA), 5.45% polyunsaturated (PUFA), mainly alpha-linolenic acid (3.9%) and linoleic acid (1.5%), and 0.04% trans fatty acids.

The NF contains also vitamins and minerals as presented in Tables 3 and 4. The presence of micronutrients highly depends on the cultivation conditions and the composition and quantity of the fertiliser used. The concentration of phylloquinone in the NF (2–12 mg/100 g) was found to be considerably higher as compared to other leafy vegetables 0.2–0.8 mg/100 g (EFSA NDA Panel, 2008).

The Panel noted that the intake of phylloquinone from the NF as food supplement, considering the range provided by the applicant (Table 6), may reach 2.4 mg/day for adults. These amounts may antagonise anticoagulants such as coumarins and for this reason consumption of the NF may constitute a risk for patients on such therapy (SCF/NDA, 2006 and EGVM, 2003).

The concentration of Mn in the NF, according to the specifications may reach 116.5 mg/kg. This concentration is higher compared to food sources rich in Mn, e.g. nuts 24.9 mg/kg; dried fruit, nuts and seeds 11.9 mg/kg; chocolate 8.9 mg/kg; bread, miscellaneous cereals 8.0 mg/kg (EFSA NDA Panel, 2013). The Panel notes that the SCF in 2000 reported that exposure to high levels of Mn by inhalation or oral intake of Mn may be neurotoxic. The SCF could, however, not set an UL for Mn and concluded that '*the margin between oral effect levels in humans as well as experimental animals and the estimated intake from food is very low. Given the findings on neurotoxicity and the potential higher susceptibility of some subgroups in the general population, oral exposure to Mn beyond the normally present in food and beverages could represent a risk of adverse health effects without evidence of any health benefit*'. (SCF/NDA, 2006).

EFSA estimated the intake of Mn from the NF, considering the product specification for Mn (Table 6) and the estimated daily intake of the NF for all population groups (Table 8). Results are presented in Table 10.

Table 10: Intake estimates of manganese resulting from the use of the NF as an ingredient in the intended food categories or as food supplement at the maximum proposed use levels

Population group	Age (years)	Mean Mn intake (mg per day)		P95th Mn intake (mg per day)	
		Lowest[(a)]	Highest[(a)]	Lowest[(b)]	Highest[(b)]
Infants	< 1	0.0255	0.1231	0.0993	0.4254
Young children[(c)]	1 to < 3	0.1724	0.3661	0.3378	0.7765
Other children	3 to < 10	0.2136	0.5140	0.4175	1.0093
Adolescents	10 to < 18	0.2049	0.4838	0.4100	1.0028
Adults[(d)]	≥ 18	0.3527	0.5818	0.6177	1.1357
From food supplements only for adults		2.33			

Mn: manganese; NF: novel food.
(a): FAIM tool exposure estimate was generated on 01/06/2021. The lowest and the highest averages observed among all EU surveys are reported in these columns.
(b): FAIM tool exposure estimate was generated on 01/06/2021. The lowest and the highest P95th observed among all EU surveys are reported in these columns (P95th based on < 60 individuals are not considered).
(c): Referred as 'toddlers' in the EFSA food consumption comprehensive database (EFSA, 2011).
(d): Includes elderly, very elderly, pregnant and lactating women.

EFSA has previously reported that estimated mean manganese intakes of adults in the EU ranged from 2 to 6 mg/day, with the majority of values around 3 mg/day. In younger age groups, mean manganese intakes in various EU countries ranged from around 1.5 to 3.5 mg/day in children, and from 2 to 6 mg/day in adolescents (EFSA NDA Panel, 2013).

The highest estimated mean intake of Mn from the NF across countries ranges between 0.1 in infants and 0.6 mg/day in adults. As compared to the highest mean background Mn intake estimates, the additional intake of manganese from the NF would be 15% for children, 8% for adolescents and 10% for adults.

The highest estimated 95th percentile intake of Mn from the NF ranges from 0.4 mg/day in infants to 1.1 mg/day in adults. As compared to the highest mean background Mn intake estimates, the additional intake of manganese from the NF would be 29% for children, 17% for adolescents and 19% for adults.

The intake of Mn from the NF as food supplement alone (2.33 mg/day) could increase Mn intake by 39% as compared to the highest background mean Mn intake estimates for adults.

The Panel considers that such an increase in Mn intake from the NF used as food ingredient or food supplements is of safety concern.

Average content of oxalic acid was provided by the applicant in nine batches of the NF, which ranges from 230 to 637 mg/100 g and found similar to commonly consumed vegetables such as spinach (700 mg/100 g), rhubarb (800 mg/100 g) and Brussels sprouts (1,500 mg/100 g) (Duke, 1992).

The Panel considers that with the exception of concerns related to the Mn intake, taking into account the composition of the NF and the proposed conditions of use, the consumption of the NF is not nutritionally disadvantageous.

3.10. Toxicological information

The applicant provided seven toxicological studies conducted with the NF. These studies which were claimed proprietary by the applicant are listed in Table 11.

Table 11: List of toxicological studies with the NF

Reference	Type of study	Test system	Dose
Study No. T-2791 (Kawamata et al., 2020)	Bacterial reverse mutation test (GLP, OECD TG 471)	*S.* Typhimurium TA98, TA100, TA1535, TA1537 and *E. coli* WP2 *uvrA*	Up to 5,000 μg/plate (absence and presence of S9 mix)
Study No. T-G383 (Kawamata et al., 2020)	*In vitro* mammalian cell micronucleus test (GLP, OECD TG 487)	Human lymphoblastoid TK6 cells	Up to 500 μg/mL [short term (absence and presence of S9 mix) and continuous treatment]
Study No. HNM-003-TOX (Pharmaseed, 2016)	Repeated dose 4-day oral toxicity study (non-GLP, inspired by OECD TG 423)	Female Sprague-Dawley rats	Up to 3,400 mg/kg bw per day
Study No. NNI001-TX01 (APS, 2018a)	Repeated dose 28-day oral toxicity study in rodents (GLP, OECD TG 407)	Wistar rats	Up to 1,200 mg NF/kg bw per day (20 g/kg feed)
Study No. NNI002-TX01 (APS, 2018b)	Repeated dose 28-day oral toxicity study in rodents (GLP, OECD TG 407)	Male Wistar rats	20 g/kg feed
Study No. B-8508 (Kawamata et al., 2020)	90-day repeated dose oral toxicity study (GLP, OECD TG 408)	Sprague-Dawley rats	Up to 13,164 mg/kg bw per day for males; 15,027 mg/kg bw per day for females
Study No. 1010 (NPHI, 2018)	90-day repeated dose oral toxicity study (non-GLP)	Wistar rats	Approximately 400–500 mg/kg bw per day

GLP: Good Laboratory Practice; OECD TG: Organisation for Economic Co-operation and Development test guidelines; APS: American Preclinical Services; NPHI: National Public Health Institute; bw: body weight.

3.10.1. Genotoxicity

The applicant submitted a bacterial reverse mutation test and an *in vitro* micronucleus test with the NF.

The bacterial reverse mutation test (proprietary data) was carried out in compliance with good laboratory practice (GLP) and following OECD Test Guideline (TG) 471 in *Salmonella* Typhimurium TA100, TA1535, TA98 and TA1537, and *Escherichia coli* WP2 *uvrA* with or without metabolic activation using the pre-incubation method. In the dose-finding test, five dose levels of the test article (Lot#2241-P) were selected (19.5, 78.1, 313, 1,250 and 5,000 μg/plate). Precipitation of the test article and no growth inhibition was observed at all dose levels in all strains with or without metabolic activation. The main test was performed twice with five dose levels of the NF between 313 and 5,000 μg/plate with or without metabolic activation. Precipitation was observed in all doses and no growth inhibition was observed in any strain, with and without metabolic activation. The NF did not induce the number of the revertant colonies up to 5,000 μg/plate, with and without metabolic activation for all strains. Increase in the number of revertant colonies in comparison with the negative control group observed in the positive control group confirmed the validity of the assay.

The *in vitro* micronucleus test (proprietary data) was carried out in compliance with GLP and following OECD Test Guideline (TG) 487 in human lymphoblastoid TK6 cells. A range-finding test was performed up to 2,000 μg/mL of the test article (Lot#2241-P) for 4-h short-term treatment with and without metabolic activation and 24-h continuous treatment without metabolic activation. There were precipitations of the culture medium at all dose levels in all treatment schedules. Precipitate interfering with observation of micronucleus specimens was observed at dose levels 667–2,000 μg/mL. Cytotoxicity was observed at 1,434 μg/mL in the short-term treatment without metabolic activation, 753 μg/mL in the short-term treatment with metabolic activation, although no cytotoxicity was observed in the continuous treatment. The main test was conducted with dose levels up to 1,000 μg/mL following the same treatment schedules as for the range-finding test. The observation of micronucleus specimens was conducted at dose levels of 125–500 μg/mL in all treatment schedules due to observed precipitates. The frequencies of micronucleated cells did not show a significant increase at any dose levels in all treatment schedules compared to the negative control group. The study was considered valid based on positive control results and historical data.

Taking into account the test results provided and considering the nature, source and production process of the NF, the Panel considers that there are no concerns regarding genotoxicity of the NF.

3.10.2. Subacute and subchronic toxicity

The applicant submitted a 4-day repeated dose study (Study No. HNM-003-TOX) which was a non-GLP study partially following OECD 423 (confidential and proprietary). Female Sprague–Dawley rats were divided into four groups (5 rats per group) and were orally administered 0, 1,700, 2,500 and 3,400 mg/kg bw of the NF per day (Lot#114P). Body weights did not differ between groups and clinical observations did not reveal any abnormal finding. Clinical chemistry and haematology analysis showed no major differences between control and dosed groups except for a highly significant reduction, about 50%, in concentrations of lactate dehydrogenase (LDH) and creatine kinase (CPK) for all test groups given the NF. No gross pathology abnormalities were observed in the animals.

A 28-day subchronic toxicity study (Study No. NNI001-TX01) was conducted in rats according to OECD TG 407 and in compliance to GLP standards (confidential and proprietary). Wistar rats (n = 6 per sex per treatment; n = 48 in total) were given a rodent diet with 5, 10 or 20 g NF/kg feed (Lot#1005P). The control group received a rodent diet without the NF. This corresponds to mean daily intake of approximately 300, 600 and 1,200 mg NF/kg bw in females and 250, 500 and 1,000 mg NF/kg bw in males, and was calculated using the terminal bw and an average daily feed consumption of 20–24 g/day per animal. No mortalities were reported during the course of the study and no statistically significant changes in body weights, feed consumption, clinical or histopathological observations were observed.

A second 28-day subchronic toxicity study (Study No. NNI002-TX01) was conducted in two groups of six male Wistar rats given a rodent diet with 20 g NF/kg feed or a diet without the NF (confidential and proprietary). Body weights, feed consumption, haematological and histopathological examination was conducted during the course of the study. No significant difference between animals administered the NF and animals given the control diet was reported.

A 90-day study (Study No. B-8508) was performed in Sprague-Dawley rats according to OECD 408 (2018) and in compliance to GLP standards (Kawamata et al., 2020-confidential and proprietary). Animals were divided into four groups (10 animals/sex per group) and fed the NF at concentrations of 0%, 5%, 10% and 20% (w/w) in a powdered basal diet for 91 days (Lot #D20190407). The mean NF intake in the 5%, 10% and 20% (w/w) groups was 3,176, 6,491 and 13,164 mg/kg bw per day for males and 3,583, 7,423 and 15,027 mg/kg bw per day for females, respectively. There were seen no treatment-related effects in the clinical observations, body weight, food consumption and ophthalmology.

Urinalysis measured through 24 h revealed a significantly increased water intake [36 mL/24 h to 45 mL/24 h (+25%)] and a significant decrease in urinary sodium [1.9 mmol/24 h to 1.3 mmol/24 h (−32%)] for the high dose males. A significant dose-related decrease in urinary sodium [1.5 mmol/24 h to 1.0 mmol/24 h in the middle dose and to 0.7 mmol/24 h in the high dose (−33% and −53%)], potassium [3.4 mmol/24 h to 2.4 mmol/24 h in the middle dose and to 2.0 mmol/24 h in the high dose (−29% and −41%)] and chloride [2.5 mmol/24 h to 1.9 mmol/24 h in the low dose, to 1.5 mmol/24 h in the middle dose and to 1.2 mmol/24 h in the high dose (−24%, 40% and −52%)] was seen in dosed females. No difference in water intake was seen for females. Urine volume was considerably lower (40–50%) in all female dose groups but this was not statistically significant. No changes in blood levels of the electrolytes in males and females were seen. There were no findings regarding weight and histopathology of the kidney in males and females. The effects in urinary excretion in females are treatment related but cannot be explained.

In haematology, a decrease in fibrinogen was recorded in middle- and high-dose females [221 mg/dL to 183 mg/dL in the middle dose and 180 mg/dL in the high dose (17% and 19%)]. The findings are considered as treatment related, but cannot be explained. No changes were seen in males and no histopathological changes in the liver were observed.

In blood chemistry, statistically significant decreases in concentrations for total cholesterol [71 mg/dL to 53 mg/dL, (−25%)], phospholipid [106 mg/dL to 80 mg/dL (−25%)], calcium [10.5 mg/dL to 10.0 mg/dL (−5%)] (also observed in low dose males) and inorganic phosphorus [6.0 mg/dL to 5.3 mg/dL (−12%)] were seen in high dose males. For all findings except Ca, the decreases were dose-related. Small but significant increases in blood concentrations of glucose, blood urea nitrogen and urea were seen in high-dose females.

A statistically significant decrease in the relative prostate weight was observed in the high-dose male group [0.28 g/100 g to 0.24 g/100 g (−14%)]. No histopathological findings were seen for prostate. Moreover, a statistically significant decrease was recorded in the relative testis weights of

middle dose males [0.76 g/100 g to 0.64 g/100 g (−16%)] and in absolute [0.94 g to 0.84 g (−11%)] and relative [0.33 g/100 g to 0.31 g/100 g (−6%)] heart weights of low dose females. Histopathological examination was carried out and no differences in incidence and severity were seen between groups for the affected organs.

A number of significant findings were seen in the urinalysis, haematology, blood chemistry and organ weights, in the animals fed with the NF. Most of these findings were considered as treatment-related, but the mode of action cannot be explained. Considered on their own, many of them will not be considered as adverse. However, taking together statistically significant outcomes especially for the high dose groups and the lack of a plausible explanation, the Panel considers the middle dose tested for males (i.e. 6.5 g/kg bw per day) as the overall no observed adverse effect level (NOAEL) of this study.

Another 90-day non-GLP study was performed in rats using three preparations of *Wolffia globosa* (NPHI, 2018-unpublished). In total 40 animals were randomly distributed into four groups (5 animals/sex per group) and received the different preparations of *Wolffia globosa* in their diets which corresponded to approximately 400–500 mg/kg bw per day. Body weights and food consumption were monitored. Histopathological examination was performed at the termination of the study with no clear evidence of test compound-related changes in any of the organs examined. Due to study design and low dose levels, this study was not considered for the overall assessment.

3.10.3. Human data

The applicant provided three human studies (Kaplan et al., 2019; Yaskolka Meir et al., 2019; Zelicha et al., 2019) that were not designed to investigate safety; nevertheless, no adverse events related to the consumption of the NF were reported.

3.11. Allergenicity

The applicant performed a literature search on the allergenic potential of *Wolffia globosa* and noted that no publications were retrieved. The applicant also referred to the history of use of water lentils in Southeast Asia and the lack of reported allergic reactions.

The Panel considers that given the protein content of the NF (40–50 g/100 g) allergic reactions to the NF are possible.

4. Discussion

The NF which is the subject of the application is *Wolffia globosa* powder. The NF is proposed to be used as food ingredient in a variety of food categories and as food supplement. The target population is the general population and exclusively adults for food supplements.

The NF is produced from *Wolffia globosa* plants which are cultivated under controlled conditions, washed and dried. The NF consists of protein, fibre, fat and micronutrients. The concentration of heavy metals, cyanotoxins and microbiological parameters as well as trace elements in the NF depends on the cultivation conditions and the fertiliser used.

The highest intake of the NF was estimated for infants at 730 mg/kg bw per day at the 95th percentile. Intake of the NF from food supplements was calculated for the target population of adults at 286 mg/kg bw per day. The Panel noted that intake of the NF as a food supplement may lead to intake of phylloquinone up to 2.4 mg/day for adults and may constitute a risk for patients on anticoagulant medication. Under the proposed uses and use levels of the NF, the intake of heavy metals and microcystins does not raise safety concerns.

Based on the compositional data presented, the intake of the NF does not lead to intake of micronutrients that exceed established upper levels (UL). However, the Panel notes that no UL for manganese has been established in the EU. SCF (2000) and SCF/NDA Panel (2006) stated that '*oral exposure to manganese beyond the normally present in food and beverages could represent a risk of adverse health effects without evidence of any health benefit'.* Consumption of the NF at the P95th will increase the highest mean dietary Mn intake by 17–29% across age groups and by 39% from food supplement in adults. This represents a substantial increase in Mn intake, which is a safety concern. Therefore, the Panel cannot conclude on the safety of the NF. The Panel notes that an assessment of an UL for Mn is ongoing (mandate No M-2021-00058).

5. Conclusions

The Panel concludes that the safety of the NF, *Wolffia globosa* powder, cannot be established.

6. Steps taken by EFSA

1) On 25/05/2020 EFSA received a letter from the European Commission with the request for a scientific opinion on the safety of *Wolffia globosa* powder as novel food. Ref. Ares(2020) 2694626 letter.
2) On 25/05/2020, a valid application on *Wolffia globosa* powder, which was submitted by name of the company, was made available to EFSA by the European Commission through the Commission e-submission portal (NF 2019/1223) and the scientific evaluation procedure was initiated.
3) On 21/07/2020, EFSA requested the applicant to provide additional information to accompany the application and the scientific evaluation was suspended.
4) On 01/10/2020 additional information was provided by the applicant through the Commission e-submission portal and the scientific evaluation was restarted.
5) On 21/10/2020, 15/01/2021, 30/03/2021, 09/06/2021, EFSA requested the applicant to provide clarifications on the information provided.
6) On 18/12/2020, 22/03/2021, 20/05/2021, 23/06/2021, additional information was provided by the applicant through the Commission e-submission portal and the scientific evaluation was restarted.
7) During its meeting on 27/10/2021, the NDA Panel, having evaluated the data, adopted a scientific opinion on the safety of *Wolffia globosa* powder as a NF pursuant to Regulation (EU) 2015/2283.

References

Appenroth KJ, 2014. Unpublished report of authentication of the plant material by Dr. Appenroth, KJ.

Appenroth KJ, Sree KS, Böhm V, Hammann S, Vetter W, Leiterer M and Jahreis G, 2017. Nutritional value of duckweeds (Lemnaceae) as human food. Food Chemistry, 217, 266–273.

APS, 2018a. Study title: GLP 28 Day Dietary Toxicology Study in Rats. Study Number: NNI001-TX01. American Preclinical Services (APS).

APS, 2018b. Study title: GLP 28 Day Dietary Toxicology Study in Rats. Study Number: NNI002-TX01. American Preclinical Services (APS).

Bhanthumnavin K and McGarry MG, 1971. *Wolffia arrhiza* as a possible source of inexpensive protein. Nature, 232, 495.

Cabrera LI, Salazar GA, Chase MW, Mayo SJ, Bogner J and Dávila P, 2008. Phylogenetic relationships of aroids and duckweeds (Araceae) inferred from coding and noncoding plastid DNA. American Journal of Botany, 95, 1153–1165.

Darragh AJ and Hodgkinson SM, 2000. Quantifying the digestibility of dietary protein. Journal of Nutrition, 130, 1850S–1856S.

Duke J, 1992. Handbook of phytochemical constituents of GRAS herbs and other economic plants, CRC Press. Agricultural Research Service, Boca Raton, FL. Available online: https://phytochem.nal.usda.gov/phytochem/search

EFSA (European Food Safety Authority), 2011. Use of the EFSA comprehensive European food consumption database in exposure assessment. EFSA Journal 2011;9(3):2097, 34 pp. https://doi.org/10.2903/j.efsa.2011.2097

EFSA (European Food Safety Authority), 2021. Technical Report on the notification of fresh plants of *Wolffia arrhiza* and *Wolffia globosa* as a traditional food from a third country pursuant to Article 14 of Regulation (EU) 2015/2283. EFSA Supporting Publication 2021;18(6):EN-6658, 15 pp. https://doi.org/10.2903/sp.efsa.2021.EN-6658

EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), 2008. Vitamin K2 added for nutritional purposes in foods for particular nutritional uses, food supplements and foods intended for the general population. EFSA Journal 2008;6(11):822, 31pp. https://doi.org/10.2903/j.efsa.2008.822

EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), 2013. Scientific Opinion on dietary reference values for manganese. EFSA Journal 2013;11(11):3419, 44 pp. https://doi.org/10.2903/j.efsa.2013.3419

EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), 2016. Guidance on the preparation and presentation of an application for authorisation of a novel food in the context of Regulation (EU) 2015/2283. EFSA Journal 2016;14(11):4594, 24 pp. https://doi.org/10.2903/j.efsa.2016.4594

EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), 2021. Safety of water lentil powder from Lemnaceae as a Novel Food pursuant to Regulation (EU) 2015/2283. EFSA Journal 2021;19(10):6845, 25 pp. https://doi.org/10.2903/j.efsa.2021.6845

EFSA Scientific Committee, 2012. Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data. EFSA Journal 2012;10(3):2579, 32 pp. https://doi.org/10.2903/j.efsa.2012.2579

EGVM (Expert Group on Vitamins and Minerals), 2003. Safe upper levels for vitamins and minerals. Available online: https://cot.food.gov.uk/sites/default/files/vitmin2003.pdf

Gilbert S, Xu J, Acosta K, Poulev A, Lebeis S and Lam E, 2018. Bacterial production of indole related compounds reveals their role in association between duckweeds and endophytes. Frontiers in Chemistry, 6, 265.

Gorissen SHM, Crombag JJR, Senden JMG, Waterval WAH, Bierau J, Verdijk LB and van Loon LJC, 2018. Protein content and amino acid composition of commercially available plant-based protein isolates. Amino Acids, 50, 1685–1695.

JIRCAS (Japan International Research Centre for Agricultural Sciences), 2010. *Wolffia globosa* (Roxburch) Den Hartog and Van der Plas (Araceae). Available online: https://www.jircas.affrc.go.jp/project/value_addition/Vegetables/105.html

Kaplan A, Zelicha H, Tsaban G, Yaskolka Meir A, Rinott E, Kovsan J, Novack L, Thiery J, Ceglarek U, Burkhardt R, Willenberg A, Tirosh A, Cabantchik I, Stampfer MJ and Shai I, 2019. Protein bioavailability of *Wolffia globosa* duckweed, a novel aquatic plant - a randomized controlled trial. Clinical Nutrition, 38, 2576–2582.

Kawamata Y, Shibui Y, Takumi A, Seki T, Shimada T, Hashimoto M, Inoue N, Kobayashi H and Narita T, 2020. Genotoxicity and repeated-dose toxicity evaluation of dried *Wolffia globosa* Mankai. Toxicology Reports, 7, 1233–1241.

Mitrovic SM, Allis O, Furey A and James KJ, 2005. Bioaccumulation and harmful effects of microcystin-LR in the aquatic plants *Lemna minor* and *Wolffia arrhiza* and the filamentous alga *Chladophora fracta*. Ecotoxicology and Environmental Safety, 61, 345.

NPHI, 2018. Study title: 91-Day Oral Toxicity Study in the Rat of Mankai Plant, Study Number: 1010. National Public Health Institute (NPHI).

Pharmaseed, 2016. Study title: safety evaluation of Mankhai following 4 days repeated dose oral administration in SD rats, Study Number: HNM-003-TOX. Pharmaseed Ltd.

Product Safety Labs, 2015. Study title: protein digestibility corrected amino acid score (PDCAAS), Study Number: 40459. Product Safety Labs.

Ramboll Environ, 2015. Report title: Generally Recognized as Safe (GRAS) Determination for Hinoman Ltd.'s Duckweed: *Wolffia globosa*. Project Number: 24-34816A. Ramboll Environ US Corporation.

Saqrane S, Ghazali IE, Ouahid Y, Hassni ME, Hadrami IE, Bouarab L, del Campo FF, Oudra B and Vasconcelos V, 2007. Phytotoxic effects of cyanobacteria extract on the aquatic plant *Lemna gibba*: microcystin accumulation, detoxication and oxidative stress induction. Aquatic Toxicology, 83, 284–294.

SCF (Scientific Committee on Food), 2000. Opinion of the Scientific Committee on Food on the Tolerable Upper Intake Level of manganese. SCF/CS/NUT/UPPLEV/21 Final, 11 pp.

SCF/NDA Panel (Scientific Committee on Food, Panel on Dietetic Products, Nutrition and Allergies), 2006. Tolerable upper intake levels for vitamins and minerals. Available online: https://www.efsa.europa.eu/sites/default/files/efsa_rep/blobserver_assets/ndatolerableuil.pdf

Shirai Y and Rambo AT, 2014. Urban demand for wild foods in northeast Thailand: a survey of edible wild species sold in the Khon Kaen municipal market. 113–130.

Siripahanakul T, Thongsila S, Tanguthong T and Chockchaisawasdee S, 2013. Product development of Wolffia-pork ball. International Food Research Journal, 20, 213–217.

UCT (University of Chemistry and Technology of) Prague, EcoMole, Dibusz K and Vejvodova P, 2020. Systematic literature search to assist EFSA in the preparatory work for the safety assessment of Novel Food applications and Traditional Food notifications. EFSA Supporting Publication 2020:EN-1774, 72 pp. https://doi.org/10.2903/sp.efsa.2019.EN-1774

Wang W, Wu Y, Yan Y, Ermakova M, Kerstetter R and Messing J, 2010. DNA barcoding of the Lemnaceae, a family of aquatic monocots. BMC Plant Biology, 10, 205.

WHO (World Health Organisation), 2007. Protein and amino acid requirements in human nutrition: report of a joint WHO/FAO/UNU expert consultation. World Health Organisation, Technical Report Series #935. Geneva, Switzerland: FAO/WHO/UNU. Available online: https://apps.who.int/iris/handle/10665/43411

WHO (World Health Organisation), 2020. Cyanobacterial toxins: microcystins. Background document for the development of WHO Guidelines for Drinking-water Quality and Guidelines for safe recreational water environments. Available online: https://apps.who.int/iris/handle/10665/338066

Yaskolka Meir A, Tsaban G, Zelicha H, Rinott E, Kaplan A, Youngster I, Rudich A, Shelef I, Tirosh A, Brikner D, Pupkin E, Sarusi B, Blüher M, Stümvoll M, Thiery J, Ceglarek U, Stampfer MJ and Shai I, 2019. A green-Mediterranean diet, supplemented with Mankai duckweed, preserves iron-homeostasis in humans and is efficient in reversal of anemia in rats. The Journal of Nutrition, 149, 1004–1011.

Zelicha H, Kaplan A, Yaskolka Meir A, Tsaban G, Rinott E, Shelef I, Tirosh A, Brikner D, Pupkin E, Qi L, Thiery J, Stumvoll M, Kloting N, von Bergen M, Ceglarek U, Blüher M, Stampfer MJ and Shai I, 2019. The effect of *Wolffia globosa* Mankai, a green aquatic plant, on postprandial glycemic response: a randomized crossover controlled trial. Diabetes Care, 42, 1162–1169.

Abbreviations

ADF	acid detergent fibre
ADME	absorption, distribution, metabolism and excretion
AOAC	Association of Official Analytical Collaboration
APS	American Preclinical Services
BAM	Bacteriological Analytical Manual
bw	body weight
°C	degree Celsius
CFR	Code of Federal Regulations
CFU	colony forming units
CON-PV	methods of Eurofins WEJ Contaminants GmbH
CPK	creatine kinase
DIN	Deutsches Institut für Normung e.V.
EDTA	ethylenediamine tetraacetic acid
EGVM	Expert Group on Vitamins and Minerals
EN	Europäische Norm (European Standards)
FAIM	Food Additive Intake Model
FAO	Food and Agriculture Organisation of the United Nations
FDA	(US) Food and Drug Administration
FG	ferrous-gluconate
GAE	gallic acid equivalents
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
GRAS	generally recognized as safe
GRIN	Germplasm Resources Information Network
HACCP	Hazard Analysis Critical Control Points
HPLC	high performance liquid chromatography
IAA	indole acetic acid
IAAs	indispensable amino acids
ICP	inductively coupled plasma
IEC	International Electrotechnical Commission
ILA	indole lactic acid
ISO	International Organization for Standardization
JIRCAS	Japan International Research Centre for Agricultural Sciences
LDH	lactate dehydrogenase
LOD	limit of detection
LOQ	limit of quantification
MED	Mediterranean diet
Mn	manganese
MPN	most probable number
MS	mass spectrometry
MUFA	monounsaturated fatty acid
NA	not available
ND	not detected
NDA	Scientific Panel On Nutrition, Novel Foods and Food Allergens
NDF	neutral detergent fibre
NF	novel food
NOAEL	No Observed Adverse Effect Level
NPHI	National Public Health Institute
OECD	Organisation for Economic Co-Operation and Development
OES	Optical Emission Spectroscopy
OMA	official method of analysis

PA	physical activity
PDCAAS	Protein Digestibility Corrected Amino Acid Score
pH	potential of hydrogen
PUFA	polyunsaturated fatty acid
PVC	polyvinyl chloride
RH	relative humidity
RI	refractive index
rRNA	ribosomal ribonucleic acid
SCF	Scientific Committee on Food
SI	Israel Standards
TDI	tolerable daily intake
UCT	University of Chemistry and Technology (Prague)
UL	Tolerable Upper Intake Level
UNU	United Nations University
USP	United States Pharmacopeia
WHO	World Health Organization
w/w	weight per weight

Appendix A – Batch to batch analysis of the amino acid profile of the NF

Parameter (%)	Batch number								Method of analysis
	#1	#2	#3	#4	#5	#6	#20	#7	
Tryptophan	0.98	1.03	0.98	1.01	1.14	1.14	0.94	1.01	AOAC 988.15
Cystine	0.45	0.42	0.46	0.46	0.48	0.48	0.55	0.45	AOAC 994.12 mod.
Methionine	0.93	0.87	0.84	0.86	0.91	0.93	0.72	0.87	AOAC 994.12 mod.
Aspartic acid	3.87	3.92	3.97	4.07	3.97	3.96	3.65	3.83	AOAC 982.30 mod.
Threonine	1.94	1.95	1.92	1.97	2.08	2.07	1.82	1.94	AOAC 982.30 mod.
Serine	1.93	1.96	1.87	1.88	2.03	2.05	1.79	1.98	AOAC 982.30 mod.
Glutamic acid	4.56	4.66	4.56	4.64	4.8	4.71	4.42	4.58	AOAC 982.30 mod.
Proline	2.06	2.09	1.98	2.04	2.15	2.18	1.75	2.06	AOAC 982.30 mod.
Glycine	2.41	2.39	2.34	2.39	2.47	2.47	2.01	2.35	AOAC 982.30 mod.
Alanine	2.76	2.6	2.56	2.61	2.87	2.85	2.35	2.74	AOAC 982.30 mod.
Valine	2.66	2.66	2.6	2.66	2.66	2.55	2.21	2.55	AOAC 982.30 mod.
Isoleucine	2.08	2.07	2.04	2.06	2.04	1.95	1.69	1.96	AOAC 982.30 mod.
Leucine	3.91	4.03	3.86	3.92	4.21	4.17	3.25	3.99	AOAC 982.30 mod.
Tyrosine	1.53	1.58	1.53	1.57	1.7	1.66	1.37	1.54	AOAC 982.30 mod.
Phenylalanine	2.48	2.51	2.4	2.45	2.62	2.58	2.27	2.40	AOAC 982.30 mod.
Total lysine	3.31	3.21	2.94	2.98	3.7	3.57	2.69	3.35	AOAC 982.30 mod.
Histidine	0.95	0.96	0.92	0.95	1	1	0.90	0.95	AOAC 982.30 mod.
Arginine	3.25	3.17	3	2.96	2.87	2.79	4.17	3.01	AOAC 982.30 mod.

AOAC: Association of Official Analytical Collaboration.

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.2021.6938#support-information-section).