Safety of water lentil powder from Lemnaceae as a Novel Food pursuant to Regulation (EU) 2015/2283.

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SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 14 September 2021

doi: 10.2903/j.efsa.2021.6845

Safety of water lentil powder from Lemnaceae 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 the safety of water lentil powder as a novel food (NF) pursuant to Regulation (EU) 2015/2283. Water lentils refer to aquatic plants belonging to the Araceae family and represented by five genera (*Lemna*, *Wolffia*, *Wolffiella*, *Landoltia* and *Spirodela*). The NF is thermally washed and dried water lentils, which are produced as a polyculture crop consisting of species from the *Lemna* genus (70–100%) and the *Wolffia* genus (0–30%). 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 expected to be stable and to comply with the specifications during the suggested shelf life. The NF is intended for human consumption as a food ingredient in herbs, spices and seasonings, sauces, soups and broths, protein products, dietary food for weight control and as a food supplement. The target population is the general population, except for food supplements which are exclusively intended for consumption by adults. The Panel considers that based on the composition of the NF and the proposed intended uses, the NF is not nutritionally disadvantageous, except for the concerns regarding intake of manganese from the NF. No adverse effect was observed in the submitted 90-day subchronic study, at the highest dose, 1,000 mg/kg body weight (bw) per day of NF. The Panel considers that, based on the protein concentration, 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, *Lemna*, *Wolffia*

Requestor: European Commission

Question number: EFSA-Q-2018-00995

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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 and 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, Steinkellner H and Knutsen HK, 2021. Scientific Opinion on the safety of water lentil powder from Lemnaceae as a Novel Food pursuant to Regulation (EU) 2015/2283. EFSA Journal 2021;19(11):6845, 25 pp. https://doi.org/10.2903/j.efsa.2021.6845

ISSN: 1831-4732

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

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1. Introduction

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

On 28 June 2017, the company Parabel Ltd submitted the novel food application for Lentein Complete to the Dutch Committee on the Safety Assessment of Novel foods (VNV Committee). The application dossier was received by the Medicines Evaluation Board (MEB) and discussed in a meeting of the VNV Committee on 31 October 2017. According to the VNV Committee, the data provided was insufficient to assess the safety of the product. Therefore, the Novel Foods Unit (NFU) of the MEB advised the applicant to revise the dossier, taking into account the issues raised by the VNV Committee for the characterization of the novel food, production process, product specifications, proposed uses and use levels and toxicological information and resubmit it under Regulation (EU) 2015/2283 for an assessment by EFSA.

On 15 November 2018, the company Parabel Ltd submitted a request to the European Commission in accordance with Article 10 of Regulation (EU) 2015/2283[1] to place water lentil powder from Lemnaceae on the Union market as a novel food (NF).

The NF is proposed for use as a food ingredient in a number of foods in different maximum levels, depending on the food category. The NF is intended for the general population. Moreover, the NF is intended for use in food supplements targeting only adults.

On 4 April 2019, in accordance with Article 10 (3) of Regulation (EU) 2015/2283, the European Commission asked the European Food Safety Authority to provide a scientific opinion on water lentil powder from Lemnaceae as a novel food, addressing all safety aspects of this food.

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 a 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: production process details including the HACCP plan and the production flow chart as well as the report on the quantification of indole-3-acetic acid (IAA) and indole-3-lactic acid (ILA) in water lentil plants and the NF by liquid chromatography–tandem mass spectrometry (LC–MS/MS).

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.

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 Dibusz and Vejvodova (2020).

[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.

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 water lentil powder. The NF is produced by cultivated members of water lentils, aquatic plants belonging to the Araceae family, and consists of protein (35–55%), fibre, fat, ash, and micronutrients. The NF is proposed to be used as an ingredient in a number of food categories and in food supplements. The target population is the general population.

The applicant indicated that, as defined by Regulation (EU) 2015/2283, Article 3, the NF falls under the category 'Foods consisting of, isolated from or produced from plants or 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'.

The assessment of the dossier was based on the data presented by the applicant in the application for authorisation of the NF in the context of Regulation (EU) 2015/2283 and the comments on the assessment of the dossier by the Dutch Committee on the Safety Assessment of Novel Foods (VNV Committee, 2017).

3.2. Identity of the NF

The NF is a whole plant powder from a combination of cultivated species commonly known as water lentils or duckweeds. Water lentils are floating aquatic plants belonging to the Lemnoideae subfamily of the Araceae family and represented by five genera (*Lemna*, *Wolffia*, *Wolffiella*, *Landoltia* and *Spirodela*) (Cabrera et al., 2008). They are small and fast-growing monocotyledonous plants with similar morphological characteristics, hindering the differentiation between several of the 38 existing species reported up to date (Wang et al., 2010). Water lentils are distributed across the globe except from polar regions and deserts; however, distribution of species varies according to the geographical region (Goopy and Murray, 2003). The NF is produced as a polyculture crop consisting of species from *Lemna* genus (70–100%) and *Wolffia* genus (0–30%). The applicant provided a list of species that are potentially intended as plant material for the NF (Appendix A List of the water lentil species to be used for the production of the NF). However, the applicant notes that up to now the NF has been exclusively produced from a co-culture of *Wolffia globosa* and *Lemna minor* species, collected in Florida (US). The applicant, following a request from EFSA, stated that the identification of the plant material is performed based on morphological features at the species level.

3.3. Production process

According to the information provided, the NF is produced in line with Hazard Analysis Critical Control Points (HACCP) principles and Safe Quality Food (SQF) program regarding the manufacturing process of the NF post harvesting.

The production process of the NF initiates with the cultivation of the plant material. Open field hydroponic lined water basins are used above the soil to prevent any lixiviation and contamination from and to the surrounding ground. The aquaculture practice involves the use of nutrients for the plants and water that is recycled. The applicant states that the use of pesticides and herbicides is excluded. The growth conditions are continuously monitored to attain optimum level of nutrients for the plants and water and to control contaminants. The plant material is harvested and transported to the processing facility. The main steps of the manufacturing process include initially the pre-screening of the fresh material to remove any foreign material followed by a thermal washing step to reduce the microbial load. The material is further dried until a low moisture content is obtained and desirable water activity levels are reached (< 0.6) to avoid growth of spoilage microorganisms. Finally, the dried material is milled to meet required particle size according to the specifications set, under controlled environment. The applicant notes that daily testing of batches is performed to ensure stable production, including microbiological parameters (aerobic plate count, yeast/mould, coliforms, *Salmonella*, *Escherichia coli*, *Listeria*, and *Clostridium perfringens*), ash, moisture, protein, fat, fibre and heavy metals. The final powder is packed into 20 kg multilayer food-grade foil bags and stored into carton boxes in a designated warehouse under controlled conditions.

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

3.4. Compositional data

The NF consists primarily of protein and fibre with lesser constituents being fat, ash, and micronutrients. The macronutrient composition of water lentils is demonstrated to depend on growth conditions and chemical composition of water irrespective of the taxonomic species (Culley et al., 1981; Appenroth et al., 2017). Thus, according to the applicant, the chemical composition of the *Wolffia* and *Lemna* species intended to be used to produce the NF is expected to be similar, as they are grown under identical growth conditions. Nevertheless, the degree of accumulation of several components, including heavy metals, micronutrients and trace elements to a certain extent depends on the plant species (Appenroth et al., 2018).

To confirm that the manufacturing process is reproducible and adequate to produce a product with the required characteristics on a commercial scale, the applicant provided analytical information for six independent batches of the NF consisting exclusively of *L. minor* (L) and *W. globosa* (W) species (Table 1). Based on literature data reporting the potential bioaccumulation of trace elements and heavy metals in water lentils (Boonyapookana et al., 2002; Hegazy et al., 2009; Megateli et al., 2009; Appenroth et al., 2017), analytical data on trace elements, heavy metals and potential contaminants originating from the cultivation conditions were provided for independent batches of the NF. In a second stage, the applicant modified the fertiliser composition and provided analytical data for two additional batches (Table 2). Species percentages of the NF were also provided.

Table 1: Batch-to-batch analyses of the NF

Parameter	Units	Method of analysis	Batch number					
			#1	#2	#3	#4	#5	#6
Species identity *L. minor* (L) *W. globosa* (W)	%	Morphological analysis		75 (L) 25 (W)	82.3 (L) 17.7 (W)	74.4 (L) 25.6 (W)	89 (L) 11 (W)	87 (L) 13 (W)
Chemical parameters								
Moisture	%	AOCS Ba 2a-38	2.47	5.61	2.86	5.51	1.60	2.33
Crude protein	%	AOAC 990.03	46.17	41.93	43.13	41.30	45.26	42.92
Crude fat	%	AOAC 922.06	9.28	7.70	9.09	9.12	9.71	8.91
Ash	%	AOAC 923.03/ 32.1.05 16th Ed.	5.03	4.95	5.11	4.52	4.34	4.11
Carbohydrates	%	By difference[(a)]	NA	3.9	3.4	3.5	5.7	10.5
Dietary fibre (total)	%	AOAC 991.43	34.2	35.9	36.4	36.1	33.4	31.2
Soluble	%	AOAC 991.43	0.7	4.5	2.5	3.5	2.3	5.6
Insoluble	%	AOAC 991.43	33.5	31.4	33.9	32.6	31.1	25.6
Minerals (micronutrients, trace elements and heavy metals)								
Potassium	mg/kg	AOAC 993.14 Mod.	4,950	3,670	2,470	3,160	4,860	3,820

Parameter	Units	Method of analysis	Batch number					
			#1	#2	#3	#4	#5	#6
Sodium	mg/kg	AOAC 993.14 Mod.	1,680	2,320	1,200	1,340	1,660	1,340
Magnesium	mg/kg	AOAC 993.14 Mod.	3,430	3,460	3,890	3,430	3,200	3,090
Aluminium	mg/kg	AOAC 993.14 Mod.	3.2	< 4.0	3.9	3.0	3.1	2.0
Arsenic	mg/kg	AOAC 993.14 Mod.	0.047	0.08	0.044	0.037	0.038	0.041
Boron	mg/kg	AOAC 993.14 Mod.	1,080	1,000	1,110	947	663	779
Cadmium	mg/kg	AOAC 993.14 Mod.	0.011	< 0.01	0.014	0.01	0.006	0.006
Calcium	mg/kg	AOAC 993.14 Mod.	13,300	13,600	17,800	15,600	11,500	13,200
Chromium	mg/kg	AOAC 993.14 Mod.	0.334	< 0.200	0.888	0.118	< 0.200	0.177
Cobalt	mg/kg	AOAC 993.14 Mod.	< 0.050	< 0.050	< 0.050	< 0.050	< 0.050	< 0.050
Copper	mg/kg	AOAC 993.14 Mod.	2.27	2.74	2.79	1.95	3.63	2.88
Iron	mg/kg	AOAC 993.14 Mod.	358	301	348	237	525	361
Lead	mg/kg	AOAC 993.14 Mod.	0.011	< 0.02	0.014	0.009	0.016	0.01
Manganese	mg/kg	AOAC 993.14 Mod.	333	128	263	157	322	223
Mercury	mg/kg	AOAC 993.14 Mod.	< 0.015	< 0.01	< 0.015	< 0.015	< 0.015	< 0.015
Molybdenum	mg/kg	AOAC 993.14 Mod.	0.100	0.104	0.076	0.066	0.122	0.071
Phosphorus	mg/kg	AOAC 993.14 mod	5780	4660	5810	4670	5120	4730
Selenium	mg/kg	AOAC 993.14 mod	< 0.30	0.17	< 0.30	< 0.30	< 0.30	< 0.30
Tin	mg/kg	AOAC 993.14 mod	< 0.15	NA	< 0.15	< 0.15	< 0.15	< 0.15
Zinc	mg/kg	AOAC 993.14 mod	56.6	40.2	71.1	57	100	76.4
Antinutrients								
Oxalic Acid	%	AOAC 986.13 mod	0.037	0.057	0.032	NA	NA	0.026
Microbiological parameters								
Aerobic plate count	CFU/g	AOAC 990.12	420,000	58,000	54,000	47,000	26,000	120,000
Salmonella spp.	Presence/ Absence per g	AOAC 2003.09	ND	ND	ND	ND	ND	ND
E. coli	MPN/g	FDA BAM Chapter 4	> 1,100	< 3 (method: AOAC 988.19)	< 3	< 3	< 3	< 3
Listeria spp.	Presence/ Absence in 25 g	AOAC-RI 050903	ND	ND	ND	ND	ND	ND

Parameter	Units	Method of analysis	Batch number					
			#1	#2	#3	#4	#5	#6
Clostridium perfringens	CFU/g	AOAC 976.30	< 10	< 10	< 10	< 10	< 10	< 10
Coliforms	CFU/g	AOAC 991.14	< 10	100	< 10	< 10	< 10	< 10
Yeasts	CFU/g	FDA BAM Chapter 18	4,500	< 10	10	40	< 10	20
Moulds	CFU/g	FDA BAM Chapter 18	< 10	< 10	10	10	< 10	< 10

AOCS: American Oil Chemists' Society; AOAC: Association of Official Analytical Collaboration; FDA: Food and Drug Administration; BAM: Bacteriological Analytical Manual; NA: Not Available; ND: Not Detected; CFU: colony forming units.
(a): The applicant calculated carbohydrates as 100% – [protein % + moisture % + fat (AH) % + ash % + dietary fibre %], differently from Regulation (EU) No 1169/2011, in which carbohydrates include fibre.

Table 2: Trace element content of two additional batches of the NF

Parameter	Units	Method of analysis	Batch number	
			#7	#8
Aluminium	mg/kg	ICP-AES/OES (In-house)	7.3	4.1
Boron	mg/kg	ICP-AES/OES (In-house)	400.0	418.2
Copper	mg/kg	ICP-AES/OES (In-house)	1.53	1.15
Iron	mg/kg	ICP-AES/OES (In-house)	173.4	167.0
Manganese	mg/kg	ICP-AES/OES (In-house)	185.6	173.3
Molybdenum	mg/kg	ICP-AES/OES (In-house)	1.5	1.5
Zinc	mg/kg	ICP-AES/OES (In-house)	230.3	84.3

ICP-AES/OES: inductively coupled plasma atomic emission spectroscopy/optical emission spectroscopy.

Analytical data on the fatty acid profile of the NF were presented for three representative batches of the NF (batches #1, 3, 6). The total fat content of the analysed batches was 6.2% w/w as triglyceride. In total, 18 individual fatty acids were measured with alpha-linolenic acid (18:3n-3) comprising 51% of the total fatty acids (%FA) followed by linoleic (18:2n-6) and palmitic (16:0) acid accounting for 17 and 16.5%FA, respectively. Total saturated fatty acids were calculated at 20.5%FA. The monounsaturated (MUFA) fatty acids were on average 2.5%FA, *cis-cis* polyunsaturated (PUFA) 74.7%FA and *trans*-fatty acids 2.3%FA.

Moreover, the applicant provided data on the concentrations of carotenoids and xanthophylls in the NF for one batch. The total reported carotenoids in the NF were 942.6 mg/kg; including 271.3 mg/kg beta-carotene. Upon request from EFSA, as the most abundant carotenoid, concentration of beta-carotene in the NF was presented in five additional representative batches and found to range between 13.4 and 271.3 mg/kg. Beta-carotene concentrations reported in the literature for *Lemna* and *Wolffia* species were collected by EFSA. Dewanji et al. (1997) reported 627 mg/kg of beta-carotene in *L. minor*, while higher amounts of beta-carotene were reported in *Lemna* species by Men et al. (1995) reaching 1,025 mg/kg. Appenroth et al. (2018) reported levels of beta-carotene in 16 *Wolffia* species ranging between 110 and 330 mg/kg.

The applicant provided analytical data on the contents of antinutritional factors (ANFs) in the NF such as oxalic acid (0.04%, Table 1), tannins (< 0.05%, expressed as catechin equivalents), trypsin inhibitors (< 1,000 TIU/g), protease inhibitors (< 0.017 U/mg) and phytic acid (0.31%). The assessment of the concentration of ANFs in the NF is provided in Section 3.9 Nutritional information.

The applicant presented analytical data on the levels of biogenic amines in the NF and noted that overall low levels of biogenic amines were detected in the NF. Putrescine was the only biogenic amine that was detected in relatively high concentrations (average levels of 62 mg/kg). The assessment of the concentration of putrescine in the NF is provided in section 3.10 Toxicological information.

Concentrations of constituents originating from the given growing conditions, namely sulfate, nitrate and nitrite were measured in one batch of the NF [nitrate 63.42 mg/kg (w/w); sodium nitrite 1.5 mg/kg (w/w); sulfates 18.4 mg/L (w/v)]. Following a request from EFSA, the applicant provided concentrations of nitrate in five additional batches of the NF and in all five batches concentration of nitrate was < 20 mg/kg. In addition, analytical data for dioxins, mycotoxins and pesticides were provided and found to be below regulatory levels established for other food categories.

Accumulation of toxins from cyanobacteria in water lentils may represent a risk to human health. In particular, microcystins have been reported to accumulate in *Lemna* and *Wolffia* species (Mitrovic et al., 2005; Saqrane et al., 2007). The potential presence of these toxins in the NF as a result of the hydroponic nature of the cultivation was assessed based on the concentrations of microcystins/nodularins, anatoxin-a and saxitoxins in the final product. Following a request from EFSA, the applicant provided analytical data on the levels of microcystins/nodularins, anatoxin-a and saxitoxins in one batch of the NF. All cyanotoxins were found to be below the detection limits of the analytical methods used (LODs: 0.007 μg/g for total ADDA microcystins/nodularins, based on the specific beta-amino acid ADDA,[3] 0.015 μg/g for anatoxin-a and 0.05 μg/g for saxitoxins).

Microbiological data were also reported by the applicant, noting that the microbiological parameters are monitored for every batch due to the nature of the growing conditions in aquaculture. The microbiological data provided for six batches (batches #1 to #6, Table 1) meet the NF product specifications except for two batches, for which the applicant indicated that the samples were contaminated, and were therefore rejected by the Quality Department (batches #1 and #6, Table 1). Following a request from EFSA, the applicant provided additional microbiological data for five representative batches of the NF (Table 3). Microbiological parameters for the additional batches were within specification limits, while reprocessing of one batch was required to meet specifications for aerobic plate counts (batch #11, Table 3).

Table 3: Additional batch-to-batch analyses of the NF

Parameter	Units	Method of analysis	Batch number				
			#9	#10	#11	#12	#13
Species Identity *L. minor* (L) *W. globosa* (W)	%	Morphological analysis	90 (L) 10 (W)	87 (L) 13 (W)	86 (L) 14 (W)	90 (L) 10 (W)	92 (L) 8 (W)
Particle size (mean diameter)	μm	Laser Diff	41.27	46.31	38.82	41.07	39.22
Chemical parameters							
Moisture	%	AOCS Ba 2a-38	4.37	5.66	6.63	2.83	3.74
Crude protein	%	AOAC 990.03	37.90	37.88	37.69	39.50	39.36
Microbiological parameters							
Aerobic plate count	CFU/g	AOAC 990.12	12,000	18,000	13,000[(a)]	5,300	12,000
Salmonella	Presence/ Absence per 25 or 375 g	AOAC 2003.09 or AOAC-RI 121501	ND per 25 g	ND per 375 g	ND per 375 g	ND per 375 g	ND per 375 g
E. coli	MPN/g	AOAC 988.19	< 3	< 3	< 3	< 3	< 3
Listeria spp.	Presence/ absence per g	AOAC-RI 050903	ND	ND	ND	ND	ND
Clostridium perfringens	CFU/g	ISO 7937	< 10	< 10	< 10	< 10	< 10
Coliforms	CFU/g	AOAC 991.14	< 100	< 100	< 100	< 100	< 100
Yeasts	CFU/g	FDA BAM Chapter 18	10	< 10	< 10	< 10	< 10
Moulds	CFU/g	FDA BAM Chapter 18	40	< 10	< 10	< 10	< 10

AOAC: Association of Official Agricultural Collaboration; ISO: International Organization for Standardization; FDA: Food and Drug Administration; MPN: most probable number; BAM: Bacteriological Analytical Manual; ND: Not Detected; CFU: colony forming units.

(a): This batch of the NF was reprocessed. The reported aerobic plate count value is taken from the reprocessed material.

[3] ADDA: (4*E*,6*E*-3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid), unusual beta-amino acid present in most (> 80%) of the known toxic penta- and heptapeptide cyanobacterial toxin congeners

Based on literature data (Kittiwongwattana and Thawai, 2015; Gilbert et al., 2018) reporting the presence of endophytic bacteria in water lentils and following a request from EFSA, the applicant investigated the occurrence of endophytic bacteria in five representative samples of the water lentils fresh crop. Eight genera of endophytic bacteria were identified in water lentils by 16S rRNA sequencing (sequence homology > 99%). Some of the identified endophytes have been associated with the production of indole alkaloids (Gilbert et al., 2018). The applicant therefore provided data on the concentration of IAA and ILA in the NF. Concentration of IAA was measured in five representative batches of the NF and found to be below the maximum level of 0.1 mg/kg FW set-out for similar foods (e.g. leaf vegetables) according to Regulation (EC) No 396/2005. ILA was measured in five representative batches of the NF and it was not detected.

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

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 four independently produced batches of the NF under accelerated conditions (#3, #4, #5 and #6) and one batch under normal conditions (batch #14). The tests at accelerated conditions were carried out at 40 °C/ 75% RH (relative humidity) for a period of 12 months. The shelf-life study under recommended storage conditions (< 25°C/50% RH) was conducted for 24 months. For both accelerated and normal conditions, the NF was packed in aluminium foil bags and the parameters measured included proximate composition, amino acid and fatty acid profile, lipid oxidation markers, pH, water activity, aerobic and yeasts and mould counts, and organoleptic attributes (visual appearance, colour and odour). Under normal conditions, all tested parameters were found stable or within specifications for a period of 24 months. Under accelerated conditions, the nutritional and microbiological stability parameters did not change significantly for 12 months. However, a significant alteration was observed beyond 3 months in accelerated conditions, in lipid oxidation markers and organoleptic parameters. High peroxide values were measured mainly for accelerated conditions after 6 months (32–230 meq/kg fat). The applicant notes that despite the high peroxide values measured, no clear trend was found in the formation of secondary lipid oxidation products such as hexanal and propanal.

Based on these data, the NF is expected to be stable for 24 months from manufacturing date, under recommended storage conditions in a packaging material that excludes light exposure.

Stability of the NF was tested in instant soup product packaged in aluminium foil bags and stored in accelerated conditions at 40°C/50% RH for 4 weeks, representing a period of 8 weeks of storage time under normal conditions according to the applicant. The soup product remained stable in terms of chemical composition; however, its colour changed significantly. Based on these data, the applicant suggested the application of modified atmosphere packaging and/or the use of antioxidants for longer shelf-life of the products.

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

3.5. Specifications

The NF is a green coloured free-flowing powder containing around 40% of crude protein. The applicant has presented the specifications of the NF which include chemical, microbiological and contaminant parameters, as indicated in Table 4. The applicant notes that daily testing of batches is performed to ensure stable production including microbiological parameters (APC, yeast/mould, coliforms, *Salmonella*, *E. coli*, *Listeria* and *C. perfringens*), ash, moisture, protein, fat, fibre and heavy metals. In cases of blending of NF batches, the final product is reanalysed.

Table 4: Specifications of the NF

Description: Green-coloured free-flowing powder	
Source: Fresh aquatic plants from *Lemna* spp. and *Wolffia* spp.	
Parameter	**Specification**
Species identity *Wolffia* (W)/*Lemna* (L)	0–30%W/70–100%L
Moisture	≤ 10%
Crude protein	35–55%
Fat	≤ 12%
Ash	< 10%
Carbohydrates	≤ 12%
Dietary fibre	30–50%
Soluble fibre	2–20%
Insoluble fibre	20–35%
Oxalic acid	< 0.1%
β-Carotene	< 300 mg/kg
Boron	< 500 mg/kg
Copper	< 5 mg/kg
Iron	< 800 mg/kg
Manganese	< 250 mg/kg
Molybdenum	< 3 mg/kg
Zinc	< 500 mg/kg
Contaminants	
Microcystins	< 0.01 mg/kg
Nitrate	< 100 mg/kg
Cadmium	< 0.05 mg/kg
Lead	< 0.1 mg/kg
Mercury	< 0.05 mg/kg
Arsenic	< 0.5 mg/kg
Microbiological parameters	
Aerobic plate count	< 20,000 CFU/g
Salmonella	ND/25 g
E. coli	< 3 MPN/g
Listeria spp.	ND/25 g
Clostridium perfringens	< 10 CFU/g
Coliforms	< 100 CFU/g
Yeasts	< 100 CFU/g
Moulds	< 100 CFU/g

CFU: colony forming units; carbohydrates: 100% – [protein % + moisture % + fat % + ash % + dietary fibre %]; ND: Not detected; MPN: Most Probable Number.

Considering the additional microbiological data provided by the applicant on aerobic plate counts for five representative batches of the NF, EFSA proposes to lower specifications for aerobic plate counts from 50,000 to 20,000 CFU/g.

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

3.6.1. History of use of the source

Water lentils or duckweed have been used for generations as animal feed. Specifically, duckweeds have been used as feed for fish, poultry, ducks and pigs (Haustein et al., 1992; Leng, 1999; Ngamsaeng et al., 2004). Besides a component of animal diets, a number of scientific articles refer to water lentils as human food resource in South Asia (Leng, 1999; Derksen and Zwart, 2010). In addition, *Wolffia arrhiza*, commonly named 'khai-nam' literally meaning 'eggs of the water' in Thai, has been traditionally consumed in Myanmar, Laos and Northern Thailand (Bhanthumnavin and McGarry, 1971). According to Appenroth et al. (2017), the plant that was widely consumed as food in Southeast Asia was *W. globosa* rather than *W. arrhiza* mentioned by Bhanthumnavin and McGarry (1971). Further investigations performed in 2016 supported the finding that the most common species found in the markets is *W. globosa* (Appenroth et al., 2017). The use of duckweeds as a food source was not extended in the rest of the world as mentioned by Iqbal (1999), due to the negative effect of the oxalic acid content on the taste and the difficulty to separate associated organisms from the original crop when grown under non-controlled conditions.

Recently, a traditional food notification regarding the consumption of *W. globosa* and *W. arrhiza* as fresh vegetable was assessed by EFSA and no safety concerns were raised (EFSA, 2021). Along with its long history as a food source in Southeast Asia, the applicant notes that *W. globosa* is recognised as an edible vegetable for humans in several databases, including the United States Department of Agriculture (USDA, 2021) Germplasm Resources Information Network (GRIN) database. Other databases classify *Lemna* and *Wolffia* species as edible plants based on book citations [e.g. Plants for future (pfaf.org), Useful Tropical Plants (tropical.theferns.info)].

3.6.2. History of use of the NF

The US Food and Drug Administration (FDA) completed the evaluation of the NF in 2018, for its intended use as a food ingredient and general protein source in food at levels ranging from 3% to 20% and concluded that the NF is GRAS under its intended conditions of use (GRN No. 742, 2018).

In addition, the applicant notes that the Food Safety Authorities of Australia and New Zealand (FSANZ) declared in January 2017 that the NF meets the definition of 'non-traditional food' on the basis that there is no history of human consumption in Australia and New Zealand, nevertheless, FSANZ following a preliminary hazard identification process concluded that although the NF is a 'non-traditional food', no safety assessment is required when the NF is used in a variety of foods up to 24 g per serving (FSANZ, 2017).

Health Canada provided a declaration in 2018 that duckweed species *W. globosa* and *L. minor* have a history of safe use as food in Canada and, therefore, the NF can be used in a variety of food categories from 1 to 24 g/serving (Health Canada, 2018).

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 excluding infants and toddlers. However, as the NF is intended to be used as an ingredient in standard food categories, it cannot be excluded that the NF would be also consumed by other groups of the population. Therefore, the safety data and the exposure assessment shall cover all population groups (Commission Implementing Regulation (EU) 2017/2469, article 5(6)). 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 a variety of food products, at the maximum use levels as indicated in Table 5. Following a request from EFSA the applicant clarified that the intended use as 'Dietary foods for weight control diets intended to replace total daily food intake or an individual meal (the whole or part of the total daily diet)' refers to meal replacement for weight control (and not total diet replacement for weight control covered by Regulation (EU) 609/2013). In addition, the applicant intends to market the NF for use in food supplements, at a maximum dose of 10 g per day.

Table 5: Food categories and maximum use levels intended by the applicant

FAIM tool codes	Food category	Max use level (mg NF/kg)
12.2	Herbs, spices, seasonings	8,000
12.5	Soups and broths	8,000
12.6	Sauces	8,000
12.9	Protein products, excluding products covered in category 1.8	21,000
13.3	Dietary foods for weight control diets intended to replace total daily food intake or an individual meal (the whole or part of the total daily diet)	90,000

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 use levels (Table 5), using the EFSA FAIM tool which is a tool for estimating chronic dietary exposure to food additives.[4] 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 intakes of the NF [on a mg/kg body weight (bw) basis], among the EU dietary surveys, are presented in Table 6.

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: Intake estimate resulting from the use of the NF as an ingredient in the intended food categories at the maximum proposed use levels

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	0.3	16.5	1.0	35.2
Young children[(d)]	1–< 3	1.4	108.7	3.5	82.1
Other children	3–< 10	0.4	49. 7	2.3	148.5
Adolescents	10–< 18	0.2	24.4	1.0	66.7
Adults[(c)]	≥ 18	0.5	19.2	1.4	50.8

NF: novel food; bw: body weight.
(a): FAIM tool exposure estimate was generated on 7/7/2021. The lowest and the highest averages observed among all EU surveys are reported in these columns.
(b): FAIM tool exposure estimate was generated on 7/7/2021. The lowest and the highest P95th observed among all EU surveys are reported in these columns (P95th based on less than 60 individuals are not considered).
(c): Including elderly, very elderly, pregnant and lactating women.
(d): Referred as 'toddlers' in the EFSA food consumption comprehensive database (EFSA, 2011).

For the intake of the NF in the form of food supplements, the proposed maximum daily doses of the NF is 10 g/day, only for adults. This corresponds to intake of 142.9 mg/kg bw per day, taking into consideration 70 kg, as the default body weight for adults (EFSA Scientific Committee, 2012).

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 considers that combined intake of the NF as food ingredient and food supplement can be expected. The combined intake of the NF as food ingredient and food supplement for adults may reach 193.7 mg/kg bw per day or 13.6 g/day, using 70 kg as the default body weight for adults (EFSA Scientific Committee, 2012).

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

3.7.5. Estimate of exposure to undesirable substances

EFSA estimated the intake of heavy metals and trace elements, which originate from the fertiliser used for the cultivation of water lentils, considering the specification levels for each element (Table 4) and the estimated daily intake of the NF for all population groups (Table 6). The Panel considers that exposure to heavy metals and most trace elements from the NF is not expected to exceed established maximum levels[5] 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 Nutritional information.

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 considering specification levels for microcystins proposed by the applicant, < 0.01 mg/kg, (Table 4) and estimated daily intake of the NF for all population groups (Table 6). The highest exposure to microcystins from the NF was calculated for children, resulting in 0.0015 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)

No ADME data have been provided for the NF.

The NF is composed of 35–55% plant protein (on DM basis), fibre/carbohydrates (30–62%) and fat (≤ 12%) which are normal constituents of a human diet. The Panel considers the constituents of the NF are expected to undergo normal metabolic processes.

3.9. Nutritional information

The NF is intended to be used as a source of protein, fibre and essential fatty acids in the human diet.

The applicant provided information on the levels of essential amino acids in the protein of the NF for one batch and compared it with amino acid levels present in various plant derived protein sources in the context of amino acid requirements in human nutrition (WHO, 2007), as shown in Table 7.

Table 7: Essential amino acid scoring

Parameter (g/100 g protein)	NF batch #14	Spinach[(a)]	Broccoli[(a)]	Soya flour[(b)]	Chickpea flour[(b)]	Scoring pattern (essential amino acid reference profiles) for adults (WHO, 2007)
Tryptophan	2.1	1.6	1.8	1.5	1.4	0.6
Threonine	3.7	4.9	3.9	4.1	3.9	2.3
isoleucine	4.4	5.9	3.9	4.2	4.1	3.0
Leucine	7.7	8.9	6.3	7.7	7.7	5.9
Lysine	6.0	7.0	7.4	6.0	7.0	4.5
Cysteine + Methionine	2.9	2.1*	1.8*	2.9	3.3	2.2
Tyrosine + Phenylalanine	8.8	5.2**	4.8**	9.0	8.6	3.8
Valine	5.3	6.5	5.7	4.4	4.2	3.9
Histidine	2.0	2.6	2.5	2.7	2.7	1.5

*: Refers exclusively to methionine.
**: Refers exclusively to phenylalanine.
(a): Edelman and Colt (2016).
(b): Jahreis et al. (2016).

The quality of the NF as protein source was assessed by Protein Digestibility Corrected Amino Acid Score (PDCAAS) which was calculated by *in vitro* digestibility method for one batch. The amino acid score for the NF was 1.02 and protein digestibility 91%, consequently the PDCAAS was calculated 0.93, with L-histidine considered as the first limiting amino acid. Based on the PDCAAS, the NF is

[5] 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.

comparable to beef (0.92; Hoffman and Falvo, 2004) and soy protein isolate (0.91; FAO/WHO, 1991). The results were drawn based on PDCAAS value obtained from one batch, and thus may not be representative of the NF.

Total dietary fibre content of the NF is 30–50%. The applicant provided analytical data on one batch regarding the fibre profile of the NF, found to be composed of 17.6% cellulose, 1.9% hemicellulose and 2.1% lignin comprising the neutral detergent fibre part of the fibre. In addition, an amount of 1.2% starch and total sugar < 0.35% was reported in the NF. The results on fibre profile of the NF obtained from one batch may not be representative of the NF.

The NF contains a total fat content of 9%, calculated as the average from six representative batches (Table 1), from which saturated fatty acids comprise 20.5% of the total fat content and polyunsaturated fatty acids 74.7%, mainly alpha-linolenic acid (51%) and linoleic acid (17%).

The NF contains also vitamins such as (the pro-vitamin) beta-carotene, alpha-tocopherol, vitamin B and minerals including boron, calcium, copper, iron, manganese, molybdenum, phosphorous and zinc. The presence of micronutrients highly depends on the cultivation conditions and the composition and quantity of the fertiliser used.

The Mn concentration in the NF could reach up to 250 mg/kg according to the product specifications, which is a high content as compared to rich sources of Mn in the diet, 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 (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 4) and the estimated daily intake of the NF for all population groups (Table 6). Results are presented in Table 8.

Table 8: Intake estimates of Mn 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/day)		P95th Mn intake (mg/day)	
		Lowest[(a)]	Highest[(a)]	Lowest[(b)]	Highest[(b)]
Infants	< 1	0.0040	0.0206	0.0013	0.0440
Young children[(c)]	1–< 3	0.0043	0.3261	0.0106	0.2462
Other children	3–< 10	0.0025	0.2869	0.0132	0.8577
Adolescents	10–< 18	0.0023	0.2649	0.0109	0.7233
Adults[(d)]	≥ 18	0.0082	0.3358	0.0248	0.8887
From food supplements only for adults		2.5			

NF: novel food.
(a): FAIM tool exposure estimate was generated on 7/7/2021. The lowest and the highest averages observed among all EU surveys are reported in these columns.
(b): FAIM tool exposure estimate was generated on 7/7/2021. The lowest and the highest P95th observed among all EU surveys are reported in these columns (P95th based on less than 60 individuals are not considered).
(c): Referred as 'toddlers' in the EFSA food consumption comprehensive database (EFSA, 2011).
(d): Including elderly, very elderly, pregnant and lactating women.

EFSA has previously reported that estimated mean Mn intakes for adults in the EU ranged from 2 to 6 mg/day, with the majority of values being around 3 mg/day (EFSA NDA Panel, 2013). In younger age groups, mean Mn 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).

Across countries, the highest estimated mean intake of Mn from the NF is about 0.3 mg/day across age groups (Table 8). As compared to the highest mean background Mn intake estimates, the additional mean intake of Mn from the NF would be 8% for children, 4% for adolescents and 6% for adults.

The highest estimated 95th percentile intake of Mn from the NF ranges from 0.25 mg/day in young children to 0.89 mg/day in adults (Table 8). As compared to the highest mean background Mn intake estimates, the additional P95th intake of Mn from the NF would be 25% for children, 12% for adolescents and 15% for adults.

The intake of Mn from the NF as food supplement alone (2.5 mg/day) could increase Mn intake by 42% 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.

According to the information provided by the applicant, the NF contains ANFs including tannins, trypsin inhibitors, oxalates and phytates. It has been reported in the literature that water lentils contain oxalates either in the free form or as oxalate crystals (Appenroth et al., 2017). The applicant provided compositional data from four representative batches showing that the NF, unlike the fresh plant source, contains low amounts of oxalic acid (0.04%). The manufacturing process of the fresh material contributes to the reduction of the levels of oxalic acid in the NF. In addition, the percentage of oxalic acid in the NF was compared to other commonly consumed plants, containing higher amounts of oxalates such as spinach 0.54% (Santamaria et al., 1999) and soybeans ranging from 0.67 to 3.5% (Massey et al., 2001). Phytic acid is known to chelate mineral ions and limiting their bioavailability and therefore considered as antinutrient (Dolan et al., 2010). The phytic acid content in the NF was reported as the average value from two representative batches (0.31%). The applicant notes that this percentage is similar or lower compared to other food sources regularly consumed by humans such as white rice (0.3%), soybeans (2.6%), cashews (2%), wheat (0.6%) and corn (0.7%) (Gilani et al., 2012). Other antinutrients including tannins, protease and trypsin inhibitors were reported in the NF at very low amounts below the detection limits of the methods used for their analysis [tannins < 0.05%, protease inhibitors < 0.17 U/mg and trypsin inhibitors < 1 TIU/g].

The Panel noted that the amounts of ANFs found in the NF are low comparing to amounts found in commonly consumed edible plants and plant products, so no safety concern should be expected regarding the antinutrient content.

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. The Panel, however, expresses safety concerns regarding Mn intake.

3.10. Toxicological information

The Panel notes that the chemical composition of water lentils mainly composed of proteins, fats and fibre (hemicelluloses and celluloses) does not raise any safety concern as such, however, many studies published in the literature report the use of water lentils to remove certain pollutants from aquatic environments such as heavy metals, metalloids and cyanobacteria toxins (Mitrovic et al., 2005; Saqrane et al., 2007; Megateli et al., 2009; Sasmaz and Obek, 2009; Dirilgen, 2011). Therefore, the conditions of cultivation of water lentils are expected to impact the toxicological profile of the NF.

For the current safety assessment of the NF, all minerals as well as contaminants including heavy metals reported by the applicant in the NF were assessed based on the maximum levels set by EU (Regulation No 1881/2006)[6] and health-based guidance values (HBGVs) set by EFSA and the Scientific Committee on Food (SCF/NDA, 2006).

In addition, the presence of putrescine was assessed in the NF. Putrescine was reported at average levels of 62 mg/kg, similar levels of putrescine have been reported in other fresh vegetables, legumes and fruits (e.g. green peppers at 90.04 mg/kg, mandarin at 90.16 mg/kg, oranges at 91.24 mg/kg; Sánchez-Pérez et al., 2018). Therefore, the presence of biogenic amines in the NF does not raise safety concerns.

The applicant provided an acute toxicity study, two genotoxicity studies and a subchronic study which were conducted with the NF in compliance with OECD principles of GLP and in accordance with OECD test guidelines No 420, 471, 474 and 408, respectively. Table 9 presents an overview of these studies.

[6] 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.

Table 9: List of toxicological studies with the NF

Test substance	Reference	Type of study	Tested population	Concentration/dose
Lentein™ complete (Lot no #15)	(Mishra, 2017)	Bacterial reverse mutation test (Ames test) (OECD 471)	Strains TA98, TA100, TA102, TA1535 and TA1537 ±S9	128, 320, 800, 2,000, 5,000 μg/plate in absence and presence of S9 mix
Lentein™ complete (Lot no #15)	(Panda, 2017)	Mammalian erythrocyte micronucleus test (OECD 474)	Swiss albino mice (5/sex per groups), 3 groups	Group I: corn oil (control group) Group II: 2,000 mg/kg bw Group III: 50 mg/kg bw (CP)
Lentein™ complete (Lot no #1)	(Pooja, 2017a)	Acute oral toxicity study (OECD 420)	1 female Wistar rat	2,000 mg/kg bw
Lentein™ complete (Lot no #1)	(Pooja, 2017b)	Repeated dose (90 days) oral toxicity study (OECD 408)	Wistar rats (10/sex per group) + (5/sex per satellite group)	0, 100, 500 and 1,000 mg/kg bw Recovery period: 0, 1,000 mg/kg bw per day of the NF, by gavage

3.10.1. Genotoxicity

Mishra (2017, unpublished) carried out a bacterial reverse mutation assay with *Salmonella* Typhimurium strains TA98, TA100, TA102, TA1535 and TA1537 with NF, suspended in DMSO (no precipitation in the top agar was observed), following OECD 471. Bacteria (with and without metabolic activation S9) were exposed to concentrations of 128, 312.5, 320, 625, 800, 1,250, 2,000, 2,500 and 5,000 μg/plate. No increase in the number of revertant colonies was observed when compared to the negative control in all strains tested, both in the absence and presence of metabolic activation.

Panda (2017, unpublished) carried out an *in vivo* mammalian erythrocyte micronucleus test (OECD 474). Thirty Swiss albino mice (5 animals per sex and group) were gavaged with 2,000 mg/kg of the NF in corn oil for two consecutive days and sacrificed 24 h after last dosing. No increases in micronucleated polychromatic erythrocytes were observed, however, toxicity to bone marrow (i.e. a decrease in the ratio of polychromatic/normochromatic erythrocytes (PCE/NCE) was not observed and, thus, systemic exposure could not be verified.

An *in vitro* micronucleus test, although recommended in the EFSA Scientific Opinion on genotoxicity testing strategies (EFSA Scientific Committee, 2011), was not provided.

Considering the results from the genotoxicity tests submitted, 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. Acute and subacute toxicity

The applicant provided an acute toxicity study in rat (Pooja, 2017a). The Panel considers that, generally, acute toxicity studies are not pertinent for the safety assessment of NFs.

3.10.3. Subchronic toxicity

Pooja (2017b, unpublished) carried out an oral 90-day study in accordance to OECD 408 with 100 Wistar rats, 20 animals per group (10 males and 10 females) treated via gavage (vehicle corn oil) with 0, 100, 500 or 1,000 mg/kg bw of the NF. Two recovery groups (5 males and 5 females) were kept for an additional 28 days. The NF (including recovery groups) did not cause effects as compared to controls up to the highest dose tested. Neither toxic signs nor symptoms and no mortality was observed in any of the groups. There were no statistically significant effects on feed consumption, body weight changes or absolute body weights. The Panel notes that the body weight between males and females were similar which is unusual and in addition that the growth curve was approximately linear and does not reach a plateau as normally expected. Haematological parameters and clinical biochemistry were not altered statistically significantly in the treated groups. Absolute and relative organ weights were not statistically significantly different to those of the control groups. Urinary analysis did not reveal changes of any parameters. No abnormalities were noticed in the ophthalmological examination. Neither external examination of rat carcasses nor internal examination of visceral body cavities revealed pathological lesions. Histopathological examination was carried out and no relevant findings were observed. Neurobehavioral examination was carried out with control and high dose groups 30 and 60 min after the last dosing before sacrifice and no significant differences between the two groups were observed. Based on the results, there were no effects observed at 1,000 mg/kg bw per day, the highest dose tested. The Panel notes that not a single parameter was affected in the course of the study in the control and treated animals, which is unusual.

3.10.4. Human data

No human studies have been provided.

3.11. Allergenicity

The applicant provided information on allergens that cross react with allergens identified in Annex II of Regulation (EU) No 1169/2011 (i.e. egg, fish, crustacean). Analytical data for one batch of the NF were provided for gluten and gluten allergen (wheat, rye, and barley) was found to be below LOD (3 mg/kg). Water lentils are consumed in Southeast Asia, however, no reports of allergenicity have been identified by a literature search performed by the applicant concerning allergenicity of *Lemna* and *Wolffia* genera. In addition, no allergenicity has been reported for the Lemnoideae subfamily and Araceae family.

The Panel considers that given the protein content of the NF (35–55 g/100 g) allergic reactions to the NF are possible.

4. Discussion

The NF, which is the subject of the application, is water lentil powder from species of *Lemna* genus (70–100%) and the *Wolffia* genus (0–30%). The NF is proposed to be used as an ingredient in a number of food categories and in food supplements. The target population is the general population and the adult population exclusively for food supplements.

The NF is produced by cultivation, washing and drying of species of *Lemna* genus (70–100%) and the *Wolffia* genus (0–30%). Of note, the applicant presented analytical data on batches produced exclusively by *L. minor* and *W. globosa* species. The NF consists of protein, fibre, fat, ash and micronutrients. The concentration of trace elements, heavy metals, cyanobacteria toxins (microcystins) and microbiological parameters in the NF highly depends on cultivation conditions of the plant material and the composition of the fertiliser used. Considering microbiological data, the Panel proposes to lower specifications for aerobic plate counts from 50,000 to 20,000 cfu/g.

The highest intake of the NF was calculated for children (3–10 years) and was ranging between 2.3 and 148.5 mg/kg bw per day at the 95th percentile. Intake of the NF from food supplements calculated only for adults was 142.9 mg/kg bw per day. 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 Mn has been established in EU. The SCF/NDA 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 95th percentile will increase the highest mean dietary Mn intake by 12–25% across age groups and by 42% when used as 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 on-going (mandate No M-2021-00058).

5. Conclusions

The Panel concludes that the safety of the NF, water lentil powder, cannot be established.

6. Steps taken by EFSA

1) On 4/4/2019 EFSA received a letter from the European Commission with the request for a scientific opinion on the safety of water lentil powder Ref. Ares(2019)2383178.
2) On 4/4/2019, a valid application on water lentil powder, which was submitted by Parabel Nutrition, Inc., was made available to EFSA by the European Commission through the

Commission e-submission portal (NF 2018/0430) and the scientific evaluation procedure was initiated.
3) On 18/7/2019, 14/11/2019 and 1/6/2021 EFSA requested the applicant to provide additional information to accompany the application and the scientific evaluation was suspended.
4) On 27/9/2019, 23/3/2020 and 6/7/2021 additional information was provided by the applicant through the Commission e-submission portal and the scientific evaluation was restarted.
5) On 8/4/2020, 4/9/2020, 4/12/2020, 8/2/2021, EFSA requested the applicant to provide clarifications on the information provided.
6) On 18/8/2020, 21/11/2020, 18/1/2021 and 5/5/2021 additional informations were provided by the applicant through the Commission e-submission portal and the scientific evaluation was restarted.
7) During its meeting on 14/9/2021, the NDA Panel, having evaluated the data, adopted a scientific opinion on the safety of water lentil powder from Lemnaceae as a NF pursuant to Regulation (EU) 2015/2283.

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Abbreviations

ADDA	(4*E*,6*E*-3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid)
ADME	absorption, distribution, metabolism and excretion
ANF	antinutritional factor
ANZ	Australia and New Zealand
AOAC	Association of Official Analytical Collaboration
AOCS	American Oil Chemists' Society
BAM	Bacteriological Analytical Manual
bw	body weight
CFU	colony forming units
DM	dry matter
DMSO	dimethylsulfoxyde
DW	dry weight
FA	fatty acid
FAIM	Food Additive Intake Model
FAO	Food and Agriculture Organization of the United Nations
FDA	(US) Food and Drug Administration
FSANZ	Food Safety Authorities of Australia and New Zealand
FW	fresh weight
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
GRAS	Generally Recognized As Safe
GRIN	Germplasm Resources Information Network
HACCP	Hazard Analysis Critical Control Points
HBGV	health-based guidance value
IAA	indole acetic acid
ICP-AES/OES	inductively coupled plasma atomic emission spectroscopy/optical emission spectroscopy
IEC	International Electrotechnical Commission
ILA	indole lactic acid
IPNI	International Plants Names Index
ISO	International Organization for Standardization
LC–MS/MS	liquid chromatography–tandem mass spectrometry
LOD	limit of detection
LOQ	limit of quantification
MEB	Medicines Evaluation Board
MPN	most probable number
MUFA	monounsaturated fatty acid
NA	not available
NCE	normochromatic erythrocytes
ND	not detected
NDA	Scientific Panel On Dietetic Products, Nutrition And Allergies
NF	novel food
NFU	Novel Food Unit
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Co-Operation and Development
PCE	polychromatic erythrocytes
PDCAAS	Protein Digestibility Corrected Amino Acid Score
pH	potential of hydrogen
PUFA	polyunsaturated fatty acid
RH	relative humidity

SCF	Scientific Committee on Food
SQF	Safe Quality Food
TDI	tolerable daily intake
TIU	trypsin inhibitor unit
UL	tolerable upper intake level
UNU	United Nations University
USDA	United States Department of Agriculture
VNV	Vereniging Nederlandse Verkeersvliegers (Dutch Committee on the Safety Assessment of Novel Foods)
WCSP	World Checklist of Selected Plant Families database
WHO	World Health Organization
w/v	weight per volume
w/w	weight per weight

Appendix A – List of the water lentil species to be used for the production of the NF

Family	Genus	Species	Authors hip	WCSP ID	IPNI ID	Publication	Collation	Date
Araceae	*Lemna*	*aequinoctialis*	Welw.	109276	526159-1	Apont.	578	1859
Araceae	*Lemna*	*gibba*	L.	109294	526178-1	Sp. Pl.	970	1753
Araceae	*Lemna*	*japonica*	Landolt	109298	526183-1	Veröff. Geobot. Inst. ETH Stiftung Rübel Zürich	70:23	1980
Araceae	*Lemna*	*minor*	L.	109308	526194-1	Sp. Pl.	970	1753
Araceae	*Lemna*	*minuta*	Kunth	109312	526198-1	Nov. Gen. Sp.	1:372	1817
Araceae	*Lemna*	*obscura*	(Austin) Daubs	109317	137290-2	Illinois Biol. Monogr.	34:20	1965
Araceae	*Lemna*	*turionifera*	Landolt	109340	526224-1	Aquatic Bot.	1:355	1975
Araceae	*Lemna*	*valdiviana*	Phil.	109342	279392-2	Linnaea	33:239	1864
Araceae	*Wolffia*	*arrhiza*	(L.) Horkel ex Wimm.	214819	1044795-2	Fl. Schles. ed. 3	140	1857
Araceae	*Wolffia*	*brasiliensis*	Wedd.	214823	287070-2	Ann. Sci. Nat., Bot. III	12:170	1849
Araceae	*Wolffia*	*columbiana*	H.Karst.	214825	526266-1	Bot. Unters.	1:103	1865
Araceae	*Wolffia*	*globosa*	(Roxb.) Hartog & Plas	214835	1135607-2	Blumea	18:367	1970

Source: World Checklist database of Selected Plant Families WCSP (https://wcsp.science.kew.org); International Plant Names Index IPNI (https://www.ipni.org).

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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.2021.6845#support-information-section).

Annex B – Batch-to-batch analysis of the NF- amino acid profile

Parameter	Units	Method of analysis	Batch number			
			#1	#2	#3	#4
Amino acid profile						
Aspartic Acid	%	AOAC 994.12	3.41	3.15	3.20	3.23
Threonine	%	AOAC 994.12	1.66	1.54	1.55	1.58
Serine	%	AOAC 994.12	1.58	1.43	1.47	1.50
Glutamic Acid	%	AOAC 994.12	4.08	3.78	3.84	3.86
Proline	%	AOAC 994.12	1.57	1.49	1.47	1.50
Glycine	%	AOAC 994.12	1.75	1.64	1.63	1.66
Alanine	%	AOAC 994.12	2.10	1.97	1.97	2.00
Valine	%	AOAC 994.12	2.23	2.07	2.09	2.11
Isoleucine	%	AOAC 994.12	1.82	1.70	1.71	1.73
Leucine	%	AOAC 994.12	3.32	3.10	3.10	3.17
Tyrosine	%	AOAC 994.12	1.53	1.42	1.42	1.44
Phenylalanine	%	AOAC 994.12	2.19	2.05	2.04	2.10
Lysine	%	AOAC 994.12	2.57	2.32	2.41	2.43
Histidine	%	AOAC 994.12	0.84	0.81	0.79	0.80
Arginine	%	AOAC 994.12	2.52	2.22	2.35	2.34
Total Amino Acids after protein hydrolysis	%	AOAC 994.12	33.17	30.67	31.03	31.45
Cysteine	%	AOAC 985.28	0.45	0.43	0.42	0.43
Methionine	%	AOAC 985.28	0.80	0.76	0.76	0.78
Tryptophan	%	AOAC 988.15	1.05	1.00	0.99	1.03

AOAC: Association of Official Analytical Collaboration.