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Safety of dried whole cell *Euglena gracilis* 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 dried whole cell *Euglena gracilis* as a novel food (NF) pursuant to Regulation (EU) 2015/2283. *E. gracilis* is a single-cell microalga which occurs widely in nature and is commonly found in freshwater habitats. The NF, the dried biomass of *E. gracilis*, is produced by fermentation and its major constituent (> 50%) is a β-glucan polysaccharide. The applicant proposed to use the NF in food supplements, in foods for total diet replacement for weight control and as a food ingredient added to a number of food products. The target population proposed by the applicant is the general population, except for food supplements and for foods for total diet replacement for which the target population is the general population from 12 months of age onwards. In 2019, *E. gracilis* was attributed the qualified presumption of safety (QPS)-status with the qualification 'for production purposes only', which includes food products based on microbial biomass of the microalga. Based on the information provided, *E. gracilis* is not expected to survive the manufacturing process. The submitted toxicity studies did not raise safety concerns. No adverse effects were observed in the subchronic toxicity study, up to the highest dose tested, i.e. 3,300 mg NF/kg body weight, considered as the no observed adverse effect level (NOAEL). The margins of exposure between this dose and the high (95th percentile) intake estimates, range from 33 for infants to 192 for adults. The Panel considers that in view of the QPS status of the source of the NF, supported by the compositional data and lack of toxicity observed in the 90-day study, the margins of exposure are sufficient. The Panel considers that the NF, i.e. dried whole cell *Euglena gracilis*, is safe at the proposed uses and use levels.

Keywords: Novel foods, *Euglena gracilis*, consumption, safety

Requestor: European Commission

Question number: EFSA-Q-2019-00043

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1. Introduction

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

On 20 December 2018, the company Kemin Foods L.C. submitted a request to the European Commission in accordance with Article 10 of Regulation (EU) No 2015/2283[1] to place dried whole cell Euglena on the European Union market as a novel food.

The novel food is proposed for use in a number of food categories and is intended for the general population.

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 dried whole cell Euglena.

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. Data and methodologies

2.1. Data

The safety assessment of this novel food (NF) is based on data supplied in the application and information submitted by the applicant following an EFSA request for supplementary information.

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 to supporting the safety of the proposed 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: *in vitro* fermentation studies (Kemin Corporation, 2016), bacterial reverse mutation assay (Product Safety Labs, 2015a), *in vivo* micronucleus test (Product Safety Labs, 2015b), acute toxicity study (Product Safety Labs, 2014), 14-day toxicity/palatability study (Product Safety Labs, 2015c), 90-day toxicity study (Product Safety Labs, 2015d).

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.

This assessment concerns only 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 that is the subject of the application is dried whole cell Euglena, which is the dried biomass of the microalga *Euglena gracilis*.

The NF falls under Article 3(2)(a)(ii) foods consisting of, isolated from or produced from microorganisms, fungi or algae, as defined in Regulation 2015/2283.

[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.

The NF is produced by fermentation and its major constituent (> 50%) is a β-glucan polysaccharide. The NF is proposed by the applicant to be used as a food supplement, in foods for total diet replacement for weight control (as defined by Regulation (EU) 609/2013[3]) and as a food ingredient in a number of foods. The target population proposed by the applicant is the general population, except for food supplements and for foods for total diet replacement for which the target population is the general population from 12 months of age onwards.

3.2. Identity of the NF

The NF is dried whole cell Euglena, which is the dried biomass of *Euglena gracilis*.

E. gracilis is a single-cell microalga, belonging to the genus *Euglena*, which are phototrophic euglenoid flagellates. *E. gracilis* occurs widely in nature and is commonly found in freshwater habitats, especially in shallow eutrophic ponds.

E. gracilis can be cultivated under a variety of conditions including autotrophically with CO_2 and light as the sole source of carbon and energy, mixotrophically in light with an organic carbon source, or heterotrophically in the dark with a carbon source (Krajčovič et al., 2015). The major energy storage in *E. gracilis* is made through production of paramylon, a β-1,3-glucan, and wax esters. *Euglena* can accumulate large amounts (i.e. up to 95% of the cell mass) of paramylon, a β-1,3-polymer of glucose, when grown in the presence of adequate carbon sources under heterotrophic growth conditions (Barsanti et al., 2011).

The specific strain used by the applicant is *E. gracilis* Klebs var. *bacillaris* ATCC (American Type Culture Collection) PTA-123017. The identity was verified with three separate cultivation lots of the algae. Identification was performed by amplifying specific gene regions via polymerase chain reaction (PCR) and comparing these sequences to the HERB™ reference DNA-sequence database. The analyses were conducted at an accredited laboratory and respective certificates of analysis were provided.

The full taxonomic classification of the employed strain is the following. Empire: Eukaryota; Kingdom: Protozoa; Subkingdom: Eozoa; Phylum: Euglenozoa; Subphylum: Euglenoida; Class: Euglenophyceae; Order: Euglenales; Family: Euglenaceae; Genus: *Euglena*; Species: *Euglena gracilis* Klebs var. *bacillaris*; Strain: ATCC PTA-123017.

The strain is deposited in the ATCC Patent Depository and the certificate of deposit was provided by the applicant.

Throughout the application dossier (including certificates of analysis and safety studies) various names are used for the NF, i.e. 'dried whole cell Euglena (WCE)', 'BetaVia™ Complete' and 'dried algae (Euglena gracilis)'. The applicant confirmed that these denominations refer to the same food, i.e. the NF that is the subject of this application.

3.3. Production process

According to the information provided, the NF is produced following current Good Manufacturing Practices (cGMP) and Hazard Analysis Critical Control Points (HACCP) principles.

The parent cell line of the *E. gracilis* strain used for the manufacturing process is maintained on agar plates stored in cool conditions in the dark. At regular intervals the algae are transferred to new plates and, as needed, to shake flasks. The reason being that microalga, including *E. gracilis*, are recalcitrant to cryogenic preservation (Day et al., 2007), and the most common procedure for conservation of microalgal cultures is perpetual maintenance (i.e. continuous culture) under controlled conditions (Lorenz et al., 2005).

For the manufacturing process of the NF, *E. gracilis* cells are transferred from the maintenance culture to shake flasks of increasing size and, subsequently, to a production fermenter, which is maintained at specified culture conditions (confidential information). A complete list of the culture media and processing aids/additives plus the respective certificates of analysis were provided (confidential information).

At the production fermenter scale, *E. gracilis* can be harvested daily. Since the process is continuous, *Euglena* may be harvested at any time (usually when a certain cell density (confidential) is

[3] Regulation (EU) No 609/2013 of the European Parliament and of the Council of 12 June 2013 on food intended for infants and young children, food for special medical purposes, and total diet replacement for weight control and repealing Council Directive 92/52/EEC, Commission Directives 96/8/EC, 1999/21/EC, 2006/125/EC and 2006/141/EC, Directive 2009/39/EC of the European Parliament and of the Council and Commission Regulations (EC) No 41/2009 and (EC) No 953/2009. OJ L 181, 29.6.2013, p. 35.

achieved). The culture is harvested by filtration methods using appropriate food grade contact materials to produce a concentrated slurry, which is then heated. After adjusting the pH of the slurry with food grade NaOH it is pumped to a drum dryer, which dries the material into flakes. The flakes are collected and subsequently milled, bagged (using appropriate food contact material) and stored until shipment.

The applicant was requested to provide evidence/information to demonstrate that *E. gracilis* is killed during the manufacturing process. In reply, the applicant reiterated that during the manufacturing process, *E. gracilis* is heated to a certain temperature which is kept for a sufficient amount of time (confidential information) to ensure that the microalgae cannot survive. To substantiate this statement, the applicant provided a reference (Khanna and Yadav, 2004) that showed that *E. gracilis* is killed after exposure to 44°C for 8 min. Furthermore, after the heat-inactivation step, the *Euglena* slurry is adjusted to a high pH (above 8), at which, according to the literature (Danilov and Ekelund, 2001), *Euglena* does not survive. Finally, the applicant emphasised that at the end of the manufacturing process, the alkaline *Euglena* slurry is heated to a temperature above 100°C, which would kill any potentially remaining viable Euglena cells.

The Panel considers that the microalga is not expected to survive the manufacturing process.

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

3.4. Compositional data

The major constituent of the NF is a β-glucan (a polymer of β-1,3-glucose), which constitutes at least 50% of the NF on a dry weight basis. This linear unbranched polymer, produced as energy storage polysaccharide by *Euglena* species, is also denominated as paramylon.

Paramylon is synthesised as a fibrillar high molecular weight polymer (≈ 500 kDa) with a high level of crystallinity (up to 90%). It is deposited in the *Euglena* cells in the form of small discoid granules. According to the literature, paramylon granules synthesised by *E. gracilis* are of high purity corresponding to 100% glucose, as measured by nuclear magnetic resonance (NMR) (Barsanti et al., 2011).

The applicant provided batch to batch testing for the content of β-glucan, organoleptic properties, heavy metals and microbial counts for five batches of the NF (Table 1).

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

Parameter (unit)	Batch number					Method of analysis
	1801111503	**1801111504**	**1801111505**	**1801111506**	**1801111509**	
Microscopic identity	Conforms	Conforms	Conforms	Conforms	Conforms	KHM-005-090
Appearance (free-flowing powder)	Conforms	Conforms	Conforms	Conforms	Conforms	KHM-005-916
Colour	Yellow-tan	Yellow-tan	Yellow-tan	Yellow-tan	Yellow-tan	KHM-005-916
Odour	Charact. of algae	Charact. of algae	Charact. of algae	Charact. of algae	Charact. of algae	KHM-005-916
β-glucan (%)[(a)]	61	62	61	61	64	AOAC 991.43
Heavy metals[(b)]						
Lead (mg/kg)	< 0.005	< 0.005	< 0.005	0.01	< 0.005	ICP-MS[(c)]
Cadmium (mg/kg)	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	ICP-MS[(c)]
Mercury (mg/kg)	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	ICP-MS[(c)]
Arsenic (mg/kg)	< 0.02	< 0.01	< 0.01	< 0.01	< 0.01	ICP-MS[(c)]
Microbiological						
Aerobic plate count (CFU/g)	7,700	5,500	8,000	6,800	1,400	AOAC 966.23
Coliforms (MPN/g)	< 3	< 3	< 3	< 3	< 3	FDA-BAM
Yeast and mould (CFU/g)	70	40	20	20	20	FDA-BAM

Parameter (unit)	Batch number					Method of analysis
	1801111503	1801111504	1801111505	1801111506	1801111509	
Escherichia coli (in 10 g)	Absent	Absent	Absent	Absent	Absent	USP
Staphylococcus aureus (in 10 g)	Absent	Absent	Absent	Absent	Absent	USP
Salmonella (in 25 g)	Absent	Absent	Absent	Absent	Absent	USP
Listeria monocytogenes (in 25 g)	Absent	Absent	Absent	Absent	Absent	AOAC 2004.06

AOAC: Association of Official Analytical Chemists; CFU: colony forming units; Charact.: characteristic; FDA-BAM: Food and Drug Administration's Bacteriological Analytical Manual; ICP-MS: inductively coupled plasma mass spectrometry; MPN: most probable number; mg/kg = parts per million; NF: novel food; USP: United States Pharmacopeia.
(a): analysed as total fibre.
(b): Residual levels of metals are tested according to a validated skip a lot testing programme.
(c): Elements by ICP-mass spectrometry (ICP-MS). Official Methods of Analysis, Method 2011.19 and 993.14, AOAC International, (modified).

The applicant also provided proximate analyses for five additional batches of the NF. Total carbohydrates ranged from 63.7% to 71.1%, of which total dietary fibre from 51.8% to 60.4%. The protein content ranged from 17.8% to 23.2%, fat from 6.1% to 9.8%, ash from 3.3% to 5.1% and moisture from 3.0% to 5.1%.

In addition to the proximates, the applicant provided detailed analyses of fatty acids, amino acids, sugars, vitamins, minerals and carotenoids in the NF.

Furthermore, analyses were provided by the applicant for polycyclic aromatic hydrocarbons (PAHs) and aflatoxins (B1, B2, G1, G2), which were all well below regulatory limits commonly used for other foods (e.g. cereal-based foods).

The applicant also investigated the toxin-producing potential of *E. gracilis*, considering that two other species, i.e. *Euglena sanguinea* and *Euglena granulate* (but not *E. gracilis*), from the same genus have been reported to be able to produce toxic secondary metabolites (Zimba et al., 2004, 2010). The applicant submitted a certificate of analysis for six samples of the NF which were all below the limit of detection (LOD) (i.e. < 0.1 pg/g) for euglenophycin.

Information was provided on the accreditation of the laboratories that conducted the analyses presented throughout the application.

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

3.4.1. Stability

The applicant provided information on stability testing, which was performed with four batches of the NF for up to 26 months under ambient conditions (15–25°C) and with one batch of the NF under accelerated conditions (40°C and 75% relative humidity) for up to 6 months.

The batches were analysed for moisture, protein and total dietary fibre content. In addition, the content of total carbohydrates was provided under ambient conditions, while in the testing under accelerated conditions also appearance and odour of the NF were evaluated.

There were no relevant changes in the parameters assessed at any time point. No changes in the sensory evaluation were observed. Based on the data, the applicant proposed that the NF would be stable when stored under ambient conditions in unopened, tightly sealed containers for a period of up to 2 years.

The Panel considers that the data provided sufficient information with respect to the stability of the NF for up to 2 years under ambient conditions.

3.5. Specifications

The specifications for the organoleptic, physicochemical and microbiological parameters of the NF are indicated in Table 2.

Table 2: Specifications of the NF

Description: The NF is the dried biomass of non-viable *Euglena gracilis*. The manufacturing process includes conditions such as alkaline pH and heat treatment, which kill the microalga **Appearance:** free-flowing yellow-tan powder with an odour characteristic of algae		
Parameter	**Specification**	**Method of analysis**
Total carbohydrates (%)	≤ 75	By calculation
β-glucan (%)	> 50	AOAC 991.43
Protein (%)	≥ 15	AOAC 968.06 and 992.15
Fat (%)	≤ 15	AOAC 922.06 and 954.02
Ash (%)	≤ 10	AOAC 923.03
Moisture (%)	≤ 6	AOAC 925.09 and 926.08
Heavy metals		
Lead (mg/kg)	≤ 0.5	ICP-MS[(a)]
Cadmium (mg/kg)	≤ 0.5	ICP-MS[(a)]
Mercury (mg/kg)	≤ 0.05	ICP-MS[(a)]
Arsenic (mg/kg)	≤ 0.02	ICP-MS[(a)]
Microbiological		
Aerobic plate count (CFU/g)	≤ 10,000	AOAC 966.23
Coliforms (MPN/g)	≤ 100	FDA-BAM
Yeast and mould (CFU/g)	≤ 500	FDA-BAM
Escherichia coli (in 10 g)	Negative	USP
Staphylococcus aureus (in 10 g)	Negative	USP
Salmonella (in 25 g)	Negative	USP
Listeria monocytogenes (in 25 g)	Negative	AOAC 2004.06

AOAC: Association of Official Analytical Chemists; CFU: colony forming units; FDA-BAM: Food and Drug Administration's Bacteriological Analytical Manual; ICP-MS: inductively coupled plasma mass spectrometry; MPN: most probable number; mg/kg: parts per million; NF: novel food; USP: United States Pharmacopeia.

(a): Elements by ICP-mass spectrometry (ICP-MS). Official Methods of Analysis, Method 2011.19 and 993.14, AOAC International, (Modified).

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

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

3.6.1. History of the source of the NF

The applicant identified a number of food supplements containing *E. gracilis*, which are marketed in the USA, China and Japan. The most significant market share of these products appears to be in Asia, particularly in Japan. The proposed intakes of dried *E. gracilis* from these products are in the range of 500 mg per day.

According to information provided by the applicant, *E. gracilis* also appears to be marketed as a food ingredient (mostly in Japan) in a number of food products (e.g. Euglena bars, Euglena honey and oat, Euglena pudding, whey protein products, Euglena smoothies, noodles with added Euglena, etc.).

When assessing *E. gracilis* for its suitability for the qualified presumption of safety (QPS) status, the BIOHAZ Panel identified information on food products containing *E. gracilis* marketed in Japan as cookies, cereal bars and nutritional drinks (Suzuki, 2017; EFSA BIOHAZ Panel, 2019).

3.6.2. History of the use of the NF

According to the information provided by the applicant, the NF is already sold in the USA at 500 mg/serving in the following foods: baked goods and baking mixes, beverages and beverage bases, cereal products, dairy product analogues, milk and milk products, processed fruits and fruit juices, soft candy, soup and soup mixes.

3.7. Proposed uses and use levels and anticipated intake

3.7.1. Target population

The target population proposed by the applicant is the general population, except for food supplements and for foods for total diet replacement for weight control (as defined by Regulation (EU) 609/2013) for which the target population is the general population from 12 months of age onwards.

3.7.2. Proposed uses and use levels

The applicant intends to market the NF as a food supplement, in foods for total diet replacement for weight control (as defined by Regulation (EU) 609/2013) and as a food ingredient added to a number of food products. The proposed use of the NF as a food ingredient with the respective food groups and the maximum use levels of the NF therein are indicated in Table 3.

Table 3: Proposed uses and use levels of the NF as a food ingredient

FoodEx No.	Name	Proposed food use	Max. use level (mg/100 g)
A.01.000001	Grains and grain-based products	Breakfast, granola and protein bars	625
A.01.000948	Milk and Dairy Products	Yoghurt	150
		Yoghurt Beverages	93.75
A.01.00147	Non-alcoholic beverages	Fruit Juices, Smoothies and Nectars, Vegetable Juices	117
		Fruit-Flavoured Drinks	37.5
A.01.001748	Products for special nutritional use	Meal replacement beverages	75

NF: novel food.

With regard to the use of the NF as a food supplement, the applicant proposed maximum daily amounts of 100 mg/day for toddlers (i.e. from 12 to 35 months), 150 mg/day for 'other children' (i.e. from 3 to 9 years), 225 mg/day for adolescents and 375 mg/day for adults.

For the use of the NF in foods for total diet replacement for weight control as defined by Regulation (EU) 609/2013, the applicant proposed 75 mg per meal for toddlers, 'other children' and adolescents, and 188 mg per meal for adults.

3.7.3. Anticipated intake of the NF

The applicant provided intake estimates for the NF based on two databases: (i) the EFSA Comprehensive European Food Consumption Database (EFSA, 2011) and (ii) the UK National Diet and Nutrition Survey (NDNS, 2008–2014).

As for the EFSA Comprehensive European Food Consumption Database, summary statistics of the database were used. Mean and high level intakes of the NF were calculated, the latter ones according to the High Exposure from Summary Statistics (HESS) model (Dempsey, 2018). The highest intake, on a body weight basis, was calculated for infants with an anticipated intake of up to 137.6 mg/kg bw per day for high level consumers.

Since the use of summary statistics is a known source of overestimation, the applicant performed a second exposure assessment based on the individual data of the UK National Diet and Nutrition Survey (NDNS, 2008–2014). The highest anticipated intake was calculated for toddlers, at 34 mg/kg bw per day at the 95th percentile in the population of consumers (no information available on infants in this survey).

In order to derive refined intake estimates for all the population groups under evaluation, an intake assessment was performed by EFSA based on individual data from the EFSA Comprehensive European Food Consumption Database (EFSA, 2011).

The food categories for the proposed uses of the NF were allocated to corresponding FoodEx2 categories of the EFSA Comprehensive Food Consumption Database as indicated in Table 4, taking into account information provided in the application dossier (i.e. Appendix D of Annex F) plus information provided by the applicant following a request for information.

Table 4: Food categories and use levels as applied for the refined intake estimate based on the EFSA Comprehensive Food Consumption Database

FoodEx2 code	Food category	Max. use level (mg/100 g)
A00EY	Cereal bars	630
A02NE	Yoghurt	150
A02NQ	Yoghurt drinks, including sweetened and/or flavoured variants	95
A039K	Fruit and vegetable juices and nectars	120
A0EQN	Soft drinks with minor amounts of fruit or flavours	40
A03RV	Meal replacement beverages	75

Mean and high (i.e. 95th percentile) intake estimates were calculated for infants, young children (i.e. toddlers), 'other children', adolescents, adults, elderly, very elderly, pregnant and lactating women, assuming that the foods contain the NF at the maximum proposed use levels.

The ranges of the estimated mean and high intakes (in mg/kg bw per day) among the individual EU dietary surveys for the various population groups are presented in Table 5. The group of adults includes elderly, very elderly, pregnant and lactating women. The highest intake was estimated for infants, at 100.7 mg NF/kg bw per day at the 95th percentile.

The contribution of each survey to the estimated intake for each population group is available in an excel file annexed to the scientific opinion (under 'Supporting information': https://doi.org/10.2903/j.efsa.2020.6100).

Table 5: Refined intake estimate of the NF from foods fortified with the NF at the maximum proposed use levels

Population groups	Number of surveys	Range of means (mg/kg bw per day)	Range of high (P95) intakes (mg/kg bw per day)
Infants (< 12 months)	13	0.8–24.7	3.9–100.7
Toddlers (12–35 months)	16	6.1–31.6	19.7–75.3
Other children (3–9 years)	19	5.5–23.2	17.5–48.8
Adolescents (10–17 years)	20	2.8–9.4	7.2–23.6
Adults (≥ 18 years)*	22	0.9–4.7	3.3–17.2

NF: novel food; bw: body weight.
*: Includes elderly, very elderly, pregnant and lactating women.

As for the intake of the NF in the form of food supplements, the proposed maximum daily doses of the NF would correspond to intakes of 8.3, 6.5, 5.2 and 5.4 mg/kg bw per day, respectively, when considering default body weights of 12 kg for toddlers, 23 kg for 'other children', 43 kg for adolescents (mean bw of age group of 10–14 years) and 70 kg for adults (EFSA Scientific Committee, 2012).

For the intake of the NF from foods for total diet replacement for weight control (as defined by Regulation (EU) 609/2013), assuming that the entire diet (i.e. 3 meals per day) would be replaced with products containing the NF and considering the proposed dose of 75 mg/meal for children (toddlers, 'other children' and adolescents) and 188 mg/meal for adults, this would be equivalent to a daily NF intake of 225 mg/day for children and 564 mg/day for adults. On a body weight basis, these daily intake levels would correspond to 18.8, 9.8, 5.2 and 8.1 mg/kg bw per day for toddlers, 'other children', adolescents and adults, respectively.

3.7.4. Combined intake from the NF and other sources

The applicant did not provide a combined/cumulative intake assessment of the NF from all sources. The reason being that foods containing the NF (i.e. food supplements or foods fortified with the NF) will be clearly labelled, indicating the proposed maximum daily dose for the NF and a warning that fortified foods and food supplements should not be consumed concomitantly.

No intake of the NF is expected from other sources (e.g. background diet) since there is currently no known additional source of dried *E. gracilis* within the EU.

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

The applicant commented that the NF is a complex ingredient that largely consists of fibre, lipids, proteins, carbohydrates and a number of vitamins and minerals, and that the vast majority of the constituents of the NF are normal components of the diet and would, thus, be digested and metabolised in a way similar to usual plant matter (e.g. vegetables). The Panel considers that no further ADME testing is necessary for the safety assessment of the NF.

3.9. Nutritional information

The applicant provided a nutritional analysis of the NF. As described in section 3.4, the NF has a high (i.e. at least 50%) content of β-1,3-glucan. Furthermore, the NF contains protein (about 20%), fat (about 8%), ash (about 4%) and moisture (about 4%) (means of the provided proximate analyses of five batches of the NF). The applicant also provided information on sugar profiles and concentrations of vitamins (C, E, D2, D3, K) and minerals, fatty acids, carotenoids and amino acids.

The Panel considers that 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

3.10.1. Qualified presumption of safety (QPS)

In 2019, *E. gracilis* was assessed by the EFSA Panel on Biological Hazards (BIOHAZ) for its suitability to be added to the list of QPS-recommended biological agents intentionally added to food or feed. For this purpose, the BIOHAZ Panel considered the identity, the body of knowledge and potential safety concerns of this microorganism. The literature searches performed did not provide any evidence for a safety concern for human or animal health for any use of *E. gracilis*. The BIOHAZ Panel concluded that *E. gracilis* may be recommended for the QPS list with the qualification 'for production purposes only' (EFSA BIOHAZ Panel, 2019).

In June 2018, the BIOHAZ Panel clarified that the qualification 'for production purpose only' implies the absence of viable cells of the production organism in the final product and can also be applied for food and feed products based on microbial biomass (EFSA BIOHAZ Panel, 2018).

3.10.2. Genotoxicity

The applicant submitted a bacterial reverse mutation test (Product Safety Labs, 2015a) and an *in vivo* micronucleus test (Product Safety Labs, 2015b). Both tests were published by Simon et al. (2016).

The bacterial reverse mutation test (Product Safety Labs, 2015a, unpublished study report, claimed as proprietary by the applicant) was performed in compliance with good laboratory practice (GLP) and following OECD Test Guideline (TG) 471. The test was carried out with *Salmonella* Typhimurium strains TA98, TA100, TA1535, TA1537 and *Escherichia coli* strain WP2*uvr*A in the presence or in the absence of an exogenous metabolic activation system (i.e. S9-mix). In order to evaluate the cytotoxicity of the test item (#040715-AM-1), a dose-finding test was performed with the five strains mentioned above, with and without metabolic activation. There was no sign of cytotoxicity and no precipitation of the test material was observed at any concentration tested. The main test was performed with the plate incorporation method, while the confirmatory test was performed with the pre-incubation method. Positive and negative controls were included. The NF induced no biologically relevant increase in the number of revertant colonies compared with the negative controls for all strains, in the presence and in the absence of S9-mix, up to 5,000 μg/plate.

The mammalian erythrocyte micronucleus test (Product Safety Labs, 2015b, unpublished study report, claimed as proprietary by the applicant) was performed in compliance with GLP and following OECD TG 474. The test was conducted in male and female Swiss albino (ICR) mice. In the preliminary test, the NF (#040715-AM-1) was administered (by gavage) to mice (3/sex per group) at doses of 0, 500 or 2,000 mg/kg bw per day for two consecutive days. All animals survived at the end of the study period and the highest dose tested was used in the main study. The testing results indicated no signs of cytotoxicity in the preliminary test. In the main test, the mice (5/sex per group) were administered the NF at doses of 0 or 2,000 mg/kg bw. Animals were dosed with the test material or negative control on days 1 and 2. The positive control was administered on day 2 only. Blood samples were

collected from all groups for analysis 44–48 h after treatment. For all groups, a minimum of 4,000 polychromatic erythrocytes per animal were scored for incidence of micronucleated immature erythrocytes. No test substance related effects on reticulocyte fraction, micronucleus frequency in normochromatic erythrocytes or frequency of micronucleated reticulocytes were observed. The Panel notes that there was no demonstration of exposure of the test substance to the bone marrow, and therefore, this test is considered inconclusive.

Even though an *in vitro* micronucleus test, as recommended in the EFSA Scientific Opinion on genotoxicity testing strategies (EFSA Scientific Committee, 2011), was not conducted, the Panel considers that given the nature of the NF, the production process, the QPS-status of *E. gracilis* and the results of the studies presented, there are no concerns with regard to genotoxicity of the NF.

3.10.3. Acute, subacute and subchronic toxicity

The applicant submitted one acute, one subacute (14-day) and one subchronic (90-day) toxicity study. All studies, except the 14-day study, were performed in compliance with GLP. The subacute and subchronic toxicity studies were carried out with a lot (i.e. # 040715-AM-1) of the NF that contained 58.8% β-glucan, 26.2% protein, 6.2% fat, 4.3% moisture and 2.5% ash. The applicant submitted full study reports for all the studies. The acute and the subchronic toxicity studies were published by Simon et al. (2016).

The acute oral toxicity study (Product Safety Labs, 2014, unpublished study report, claimed as proprietary by the applicant) was performed in accordance with OECD TG 402. Female nulliparous Sprague–Dawley rats (3/group) were fasted overnight and then orally administered the NF (# 051614-AM-1) at a dose of 5,000 mg/kg bw. All animals survived the test substance administration. There were no signs of gross toxicity, adverse effects, or abnormal behaviour. No gross abnormalities were noted for any of the animals when necropsied at the conclusion of the 14-day observation period.

The 14-day dietary toxicity study (Product Safety Labs, 2015c, unpublished study report, claimed as proprietary by the applicant) was based on OECD TG 407. The study was not performed in full compliance with GLP standards but was conducted in a GLP-compliant facility. The aim of the study was to assess the palatability of the NF and to identify appropriate dietary levels for the 90-day subchronic toxicity study. Sprague–Dawley rats (5/sex and group) were randomised to receive 0, 1.25, 2.5 or 5.0% of the NF (Lot #: 040715-AM-1) in the diet. There were no mortalities during the study period. As there were no changes on bodyweight, bodyweight gain, food consumption or food efficiency, it was concluded that the animals are expected to tolerate at least 5% (i.e. 50,000 mg/kg feed) of the NF in the diet.

The 90-day oral toxicity study (Product Safety Labs, 2015d, unpublished study report, claimed as proprietary by the applicant) was conducted in accordance with OECD TG 408.

Sprague–Dawley rats (10/sex and group) of 7-8 weeks of age were randomly distributed to receive 0, 1.25, 2.5 or 5.0% of the NF (Lot #: 040715-AM-1) in the diet. All animals survived to the end of the study period. There were no findings with respect to clinical observations, ophthalmology and behavioural analysis of the animals. There were no relevant differences in body weight or body weight gain, food consumption or food efficiency. No changes were found for macroscopic and microscopic observations.

No changes were seen in haematology (including blood coagulation tests) except for haematocrit, for which a statistically significant decrease was found in males in the mid-dose group only (without dose response relationship). In clinical chemistry, the only statistically significant difference observed was a decrease of serum aspartate aminotransferase (AST) in males in the high-dose group. No effects were observed for parameters of urinalysis. Concerning organ weights, the only observations were reduced weights (statistically significant) of adrenal glands and epididymis in males in the low-dose group. The Panel considers these findings as incidental.

Based on the results, the Panel considers as the no observed adverse effect level (NOAEL) the highest dose (i.e. 5%) tested in the study, equivalent to 3,300 mg NF/kg body weight per day.

3.11. Allergenicity

The Panel notes the protein content of about 20% in the NF and, therefore, the potential of the NF to elicit allergic reactions.

A comprehensive literature search performed by the applicant did not reveal any studies or case reports raising potential concerns on the allergenicity of *E. gracilis*. The applicant also pointed out the history of use of *E. gracilis* in Japan and the US and the lack of identified allergenic reactions so far.

The Panel considers that the risk of allergic reactions to the NF for the general population is unknown but expected to be low.

4. Discussion

The NF, which is the subject of the application, is the dried biomass of *Euglena gracilis*. The Panel considers that the information provided on the composition of the NF is sufficient and does not raise safety concerns.

In 2019, *E. gracilis* was assessed by the EFSA BIOHAZ Panel and attributed the QPS status with the qualification 'for production purposes', which implies the absence of viable Euglena cells in the final product and can also be applied for food products based on microbial biomass of the microalgae.

The Panel considers that based on the information provided, the microalga is not expected to survive the manufacturing process and thus, the production process does not raise safety concerns.

The applicant intends to market the NF as a food supplement, in foods for total diet replacement for weight control and as a food ingredient added to a number of food products. The target population proposed by the applicant is the general population, except for food supplements and for foods for total diet replacement for which the target population is the general population from 12 months of age onwards.

Intake estimates for the NF consumed via foods fortified with the NF were performed for all population groups, based on the EFSA Comprehensive European Food Consumption Database. The highest intake estimate was calculated for infants, at 100.7 mg NF/kg bw per day at the 95th percentile.

The submitted toxicity studies did not raise safety concerns. No adverse effects were observed in the subchronic study, up to the highest dose tested, i.e. 3,300 mg NF/kg body weight per day, which the Panel considers as the NOAEL of the study.

The margins of exposure between the NOAEL of 3,300 mg/kg bw per day, the highest dose tested, and the high (95th percentile) intake estimates, range from 33 (infants) to 192 (adults).

The Panel considers that in view of the QPS status of the source of the NF, supported by the compositional data and lack of toxicity in the experimental studies, the margins of exposure are sufficient.

5. Conclusions

The Panel considers that the NF, i.e. dried whole cell *Euglena gracilis*, is safe at the proposed uses and use levels.

The Panel could not have reached the conclusion on the safety of the NF under the proposed conditions of use without the full study-report of the 90-day toxicity study (Product Safety Labs, 2015d) for which protection of proprietary data was requested by the applicant.

6. Steps taken by EFSA

1) Letter from the European Commission to the European Food Safety Authority with the request for a scientific opinion on the safety of dried whole cell *Euglena*. Ref. Ares(2019) 3149232, dated 13/05/2019.
2) On 13 May 2019, a valid application on dried whole cell Euglena, which was submitted by Kemin Foods L.C., was made available to EFSA by the European Commission through the Commission e-submission portal (NF 2018/0669) and the scientific evaluation started.
3) On 18 July 2019, EFSA requested the applicant to provide additional information to accompany the application and the scientific evaluation was suspended.
4) On 18 September 2019, additional information was provided by the applicant and the scientific evaluation was restarted.
5) During its meeting on 25 March 2020, the NDA Panel, having evaluated the data, adopted a scientific opinion on the safety of dried whole cell *Euglena gracilis* as a NF pursuant to Regulation (EU) 2015/2283.

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Abbreviations

ADME	absorption, distribution, metabolism and excretion
AOAC	Association of Official Analytical Chemists
AST	aspartate aminotransferase
ATCC	American Type Culture Collection

BIOHAZ	Biological Hazards
bw	body weight
CFU	colony forming units
cGMP	current good manufacturing practices
Charact	characteristic
FDA-BAM	Food and Drug Administration's Bacteriological Analytical Manual
GLP	good laboratory practice
HACCP	hazard analysis critical control points
HESS	High Exposure from Summary Statistics
ICP-MS	inductively coupled plasma mass spectrometry
LOD	limit of detection
MPN	Most probable number
NDNS	National Diet and Nutrition Survey
NMR	nuclear magnetic resonance
NOAEL	no observed adverse effect level
NF	novel food
OECD	Organisation for Economic Co-operation and Development
PAH	polycyclic aromatic hydrocarbons
PCR	polymerase chain reaction
QPS	qualified presumption of safety
USP	United States Pharmacopeia
WCE	whole cell Euglena