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Safety of oil from *Schizochytrium limacinum* (strain FCC-3204) for use in food supplements 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 *Schizochytrium* sp. oil as a novel food (NF) pursuant to Regulation (EU) 2015/2283. *Schizochytrium* sp. is a single-cell microalga. The strain FCC-3204, used by the applicant (Fermentalg), belongs to the species *Schizochytrium limacinum*. The NF, an oil rich in docosahexaenoic acid (DHA), is obtained from microalgae after enzymatic lysis. The applicant proposed to increase the use level of the NF as a food supplement, from 250 mg DHA/day (currently authorised for the general population, excluding pregnant and lactating women) to 3 g DHA/day for adults, excluding pregnant and lactating women. *S. limacinum* was attributed the qualified presumption of safety (QPS) status with the qualification 'for production purposes only'. Data provided by the applicant demonstrated the absence of viable cells in the NF. No toxicological studies were performed with the NF. However, based on the available toxicological data on oils derived from *Schizochytrium* sp., the QPS status of the source of the NF, the production process, the composition of the NF and the absence of viable cells in the NF, the Panel considers there are no concerns with regard to toxicity of the NF. The Panel considers that the data provided by the applicant are not sufficient to conclude on the safety of the NF at the proposed uses (3 g DHA/day as a food supplement) in adults. However, in 2012, the Panel concluded that supplemental intakes of DHA alone up to about 1 g/day do not raise safety concerns for the general population. The Panel concludes that the NF is safe for the use in food supplements at the maximum intake level of 1 g DHA/day for the target population (adults, excluding pregnant and lactating women).

Keywords: Novel foods, *Schizochytrium limacinum*, docosahexaenoic acid (DHA), food supplements, alga, fatty acid, safety

Requestor: European Commission

Question number: EFSA-Q-2019-00323

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1. Introduction

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

Schizochytrium sp. oil is authorised, in accordance with Regulation (EC) No 258/97[1], as a novel food for a number of uses as listed in Commission Implementing Regulation (EU) 2017/2470[2] establishing the Union list of novel foods in accordance with Regulation (EU) 2015/2283[3]. On 15 April 2019, the company Fermentalg submitted a request to the European Commission in accordance with Article 10 of Regulation (EU) 2015/2283 for an extension of use of *Schizochytrium* sp. oil as a novel food. The applicant requests to authorise use of *Schizochytrium* sp. oil in food supplements, excluding food supplements for infants and young children.

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 *Schizochytrium* sp. oil.

1.2. Information on existing evaluations and authorisations

Two existing evaluations of the NDA Panel of EFSA need to be mentioned:

- In the Scientific Opinion related to the tolerable upper intake level of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and docosapentaenoic acid (DPA) (EFSA NDA Panel, 2012), the Panel concluded that a tolerable upper intake level for DHA could not be established. However, it was noted that supplemental intakes of EPA and DHA combined at doses up to 5 g/day, and supplemental intakes of EPA alone up to 1.8 g/day, do not raise safety concerns for the adult population. The Panel also considered that supplemental intakes of DHA alone up to about 1 g/day do not raise safety concerns for the general population. Limited data were available on the effects of long-term supplementation with DHA alone at higher doses.
- In the Scientific Opinion on the extension of use for DHA- and EPA-rich algal oil from *Schizochytrium* sp. as a novel food (NF) ingredient (EFSA NDA Panel, 2014), it was concluded that the use of the NF in food supplements up to a maximum DHA and EPA content of 3 g/daily dose for the adult population, excluding pregnant and lactating women was safe. It was concluded that the use of the NF in food supplements up to a maximum DHA and EPA content of 3 g/daily dose for the adult population, excluding pregnant and lactating women was safe.

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. In addition, information provided by the EFSA Panel on Biological Hazards has also been considered (EFSA BIOHAZ Panel, 2020).

Administrative and scientific requirements for NF applications referred to in Article 10 of Regulation (EU) 2015/2283 are listed in Commission Implementing Regulation (EU) 2017/2469[4].

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.[5] As indicated in this guidance, it is

[1] Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel foods ingredients. OJ L 43, 14.2.1997, p. 1.

[2] Commission Implementing Regulation (EU) 2017/2470 of 20 December 2017 establishing the Union list of novel foods in accordance with Regulation (EU) 2015/2283 of the European Parliament and of the Council on novel foods. OJ L 351, 30.12.2017, p. 72.

[3] 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.

[4] 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.

[5] EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), Turck D, Bresson J-L, Burlingame B, Dean T, Fairweather-Tait S, Heinonen M, Hirsch-Ernst KI, Mangelsdorf I, McArdle H, Naska A, Neuhäuser-Berthold M, Nowicka G, Pentieva K, Sanz Y, Siani A, Sjödin A, Stern M, Tomé D, Vinceti M, Willatts P, Engel K-H, Marchelli R, Pöting A, Poulsen M, Salminen S, Schlatter J, Arcella D, Gelbmann W, de Sesmaisons-Lecarré A, Verhagen H and van Loveren H, 2016. Guidance on the preparation and presentation of an application for authorisation of a novel food in the context of Regulation (EU) 2015/2283. EFSA Journal 2016;14(11):4594, 24 pp. https://doi.org/10.2903/j.efsa.2016.4594

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 of supporting the safety of the proposed NF.

This NF application does not include a request for the protection of proprietary data.

2.2. Methodologies

The assessment follows the methodology set out in the EFSA guidance on NF applications[5] and the principles described in the relevant existing guidance documents from the EFSA Scientific Committee. The legal provisions for the assessment are laid down in Article 11 of Regulation (EU) 2015/2283 and in Article 7 of Commission Implementing Regulation (EU) 2017/2469.

This assessment concerns only 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 the 'DHA-rich oil from *Schizochytrium* sp'. It is produced by the microalgae *Schizochytrium* sp. (strain FCC-3204). With reference to article 3 of the NF Regulation 2015/2283, the NF falls under the category 2(a)(ii): 'food consisting of, isolated from or produced from microorganisms, fungi or algae'. The production process involves the controlled growth of these algae followed by extraction and refinement of the oil produced by the algae. The oil is a mixture of triglycerides composed of polyunsaturated fatty acids (PUFA) in which DHA) represents more than 55%. The NF is proposed to be used as food supplement at 3 g DHA/day in adults, excluding pregnant and lactating women.

3.2. Identity of the NF

The NF under assessment in the present application is an oil rich in DHA. Common names to define this NF are 'DHA-rich oil from *Schizochytrium* sp.' or 'DHA-rich algal oil'. The oil produced by Fermentalg contains more than 55% DHA.

The NF is isolated from marine microalgae belonging to the genus *Schizochytrium*. The taxonomic classification of the microalgae is commonly defined as follows: Kingdom: Chromista; Phylum: Bigyra; Class: Labyrinthula; Order: Thraustochytriida; Family: Thraustochytriaceae; Genus: *Schizochytrium*.

Some databases refer to the taxonomy Eukaryota, stramenopiles instead of mentioning the Kingdom (Chromista) and the Phylum (Bigyra). Nevertheless, this is still leading to the class of Labyrinthula (https://www.uniprot.org/taxonomy/2163902). Furthermore, the taxonomic classification of the genus *Schizochytrium* has been subject to discussions in 2007 (Yokohama and Honda, 2007). Based on genetic and phenotypic analysis, the authors proposed changes in the classification. The genus *Schizochytrium* was amended and new genera such as *Aurantiochytrium* and *Oblongichytrium* were defined. Therefore, the genus *Schizochytrium* can now also be referred to as *Aurantiochytrium*.

Upon EFSA's request for information, the applicant specified that the strain used to produce the NF is *Schizochytrium* sp. FCC-3204. This strain was obtained without use of mutagenic agents or genetic modifications. 'FCC 3204' has been deposited in the Culture Collection of Algae and Protozoa (CCAP), Scottish Marine Institute (SAMS), under the reference CCAP 4062/7.

The strain FCC-3204 is a natural variant of the strain FCC-1324, which was found by the applicant after analysing the fatty acid (FA) profiles of a number of different isolates and retained because of its unusually high DHA levels. The strain FCC-1324 was assessed by the Food Safety Authority of Ireland (FSAI, 2014) and recognised as a valid source to produce the NF '*Schizochytrium* sp. oil' which is currently authorised on the Union list following the initial assessment by the UK Advisory Committee in 2002 (United Kingdom, 2002). Subsequently, following an assessment by the French Agency for Food, Environmental and Occupational Health and Safety (Anses, 2018), the DHA-rich oil produced from strain FCC-3204 was considered to be substantially equivalent to the oil from the strain FCC-1324. Therefore, it is considered that the strain FCC-3204, subject of the current application, is a valid source for the NF '*Schizochytrium* sp. oil' currently authorised on the Union list.

A phylogenetic tree of the Thraustochytriceae family has been reported by the applicant. According to the applicant, this classification is based on the comparison of sequences of the 18S small subunit

of ribosomal DNA (18S SSU-rDNA). This analysis shows that the strain FCC-1324 (parent strain of FCC-3204) is close to strain ATCC 20888, which is the source of the currently authorised '*Schizochytrium* sp. oil'.

Upon EFSA's request for information, the identity of FCC-3204 at species level was addressed by the applicant based on the comparison of the genome sequence of FCC-1324, which is the parent strain of FCC-3204, with the genome sequence of the type strain *Aurantiochytrium limacinum* ATCC MYA-1381 (equivalent to *S. limacinum* ATCC MYA-1381). According to this analysis, average nucleotide identity (ANI) between the genomes of ATCC MYA-1381 and FCC-1324 was 98.89%, and the two also have a high degree of collinearity. These data show that the strain FCC-3204 is a member of the species *S. limacinum*, which is a synonym of *Aurantiochytrium limacinum* (Morabito et al., 2020).

3.3. Production process

The unicellular microalgae *Schizochytrium* sp. (FCC-3204) are grown under controlled conditions (time, temperature, pH and aeration) in a liquid culture medium containing the necessary nutrients. The cultivation process starts in the laboratory. The production method and the control and verification processes ensure that the algae used in the production are pure cultures. The water used for the controlled growth of the microalgae and throughout the production process is food-grade and conforms to the requirements set out by EU Directive 98/83/CE. The entire process is carried out under inert atmosphere or vacuum conditions. Quality control evaluations are performed at each stage of production in accordance with a HACCP system and GMP.

The microalgal biomass is lysed directly via an enzymatic hydrolysis. This lysis involves a food-grade and non-GMO enzyme. Characteristics of this enzymatic preparation are provided by the applicant (purity, pH range, temperature activity and temperature of inactivation). The manufacturer certifies that the production strain of this enzyme is not present in the enzymatic preparation and that it does not produce toxins. The enzyme was not detected in three batches of the NF. The crude oil is recovered from the lysed biomass by centrifugation. A clarification step by filtration is performed to remove solid matter. The crude oil is subsequently refined using standard techniques (neutralization, decolouration and deodorisation at high temperature). At different steps of the process, EU authorised antioxidants are added to ensure stability. The NF is finally packaged in airtight and light-proof containers and stored at a temperature of –20°C.

The applicant provided data demonstrating that the algal strain *Schizochytrium* sp. (FCC-3204) does not produce toxins and data on the absence of viable cells in the NF.

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

3.4. Compositional data

In the application initially submitted the applicant did not provide any compositional data on the NF, this being an application for an extension of use. However, considering that this extension of use relates to an increase in the daily intake of the NF in food supplements to 3 g DHA, EFSA requested the applicant to provide data on the composition of the NF. Thus, the applicant provided analytical data for 5 independent batches of the NF (Table 1), in order to confirm that the manufacturing process is reproducible and adequate to produce a product with the required characteristics on a commercial scale. Information was provided on the accreditation of the laboratories that conducted the analyses presented in the application.

The NF consists of triglycerides composed PUFA in which DHA is the predominant one, making up together with docosapentaenoic (DPA; n-6) and palmitic acid more than 92% of the total FAs.

The proximate analytical results show that the NF is almost entirely composed of crude fat (at least 94%). The applicant provided a new analysis of 5 independent batches of the NF which reported contents of proteins below the limit of quantification (LOQ; 0.25%).

Based on the batch-to-batch analysis, the EPA:DHA ratio of the NF ranges from 0.008:1 to 0.017:1.

The batch-to-batch analysis indicates a good reproducibility of the production process. A slight variability of certain nutrients (vitamin E, β-carotene, iron) was observed. This was explained by the use of food additives and processing aids in the refining step. The concentration of total sterols varied between 9,087 and 13,375 mg/kg, which is in a similar range to that observed in different types of oils (Yang et al., 2019).

In terms of chemical contaminants, the concentrations of metals (< LOQs), polychlorobiphenyls (PCBs), dioxins and polycyclic aromatic hydrocarbons found in this batch-to-batch analysis are within the EU limits established in the respective regulations and do not present concerns from a safety point of view. Results on the process contaminants glycidyl fatty acid esters (expressed as glycidol) and total 3-MCPD (free and FA esters) were available for four batches of the NF. Levels were below the maximum levels (ML) for edible oils set in the EU regulation. The analyses on microbiological contaminants in the NF are presented in Table 1. Upon EFSA's request for information, the applicant provided analyses on *Enterobacter sakazakii,* which was not detected in five batches of the NF.

The results of analyses in three batches of the NF showed that common marine biotoxins were below the respective LOQs (diarrhetic shellfish poisoning toxins: LOQ = 20 μg/kg; pectenotoxins: LOQ = 20 μg/kg; paralytic shellfish poisoning toxins, azaspiracids, yessotoxins, saxitoxin, okadaic acid: LOQ = 5 μg/kg; domoic acid: LOQ = 1 mg/kg). These data confirm that the NF, produced from *Schizochytrium* sp. (strain FCC-3204), is not expected to contain marine biotoxins, neither produced by the source organism (for which a QPS status was concluded) nor from external contamination (considering the description of the production process in Section 3.3).

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

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

Parameter (unit)	Batch number				
	0403019	**0413022**	**0418028**	**0419022**	**0419028**
Proximate analysis					
Energy (kcal/100 g)	900	900	851	987	851
Fat (g/100 g)	> 99.22	> 99.22	> 93.78	> 99.4	> 93.78
Proteins (g/100 g)[(a)]	< 0.5[(d)]	< 0.5[(d)]	< 0.5[(d)]	0.6	< 0.5[(d)]
Carbohydrates (g/100 g)	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Crude ash (g/100 g)	< 0.25	< 0.25	< 0.25	< 0.25	< 0.25
Salt (g/100 g)	0.0135 ± 0.0131	0.0390 ± 0.0136	0.0163 ± 0.0131	< 0.01	0.0250 ± 0.0132
Sodium (g/100 g)	0.005 ± 0.005	0.016 ± 0.005	0.007 ± 0.005	< 0.005	0.011 ± 0.005
Physico-chemical parameters					
p-Anisidine value	6.1	2.0	7.3	1.6	5.6
Moisture and volatiles (%)	< 0.1[(e)]	< 0.1[(e)]	< 0.1[(e)]	< 0.1[(e)]	< 0.1[(e)]
Unsaponifiable matter (%)	1.5	1.2	1.4	1.4	1.1
Acid value (mg KOH/g)	0.4	0.2	0.2	0.4	0.5
Peroxide value (meq/kg)	1.7	0.5	1.1	2.1	1.1
Density (kg/L)	NA	0.94700	0.9452 ± 0.0008	0.9493 ± 0.0008	0.9452 ± 0.0008
Relative density	NA	0.9487	0.9469 ± 0.0008	0.9480 ± 0.0008	0.9469 ± 0.0008
Tocopherols (mg/100 g)					
α-Tocopherol	26.2	26.2	13.9	26.8	14.1
β-Tocopherol	4.06	4.01	2.05	3.12	2.13
δ-Tocopherol	85.8	74.2	39.3	69.5	38.7
γ-Tocopherol	195	171	87.5	162	86.3
Sum of tocopherols	311	276	143	262	141
Carotenoids					
β-Carotene (μg/100 g)	NA	< 5	49.1 ± 13.7	< 5	< 5

Parameter (unit)	Batch number				
	0403019	0413022	0418028	0419022	0419028
Lutein and zeaxanthin (mg/100 g)	NA	< 2	< 2	< 2	< 2
Astaxanthin (mg/100 g)	NA	< 2	< 2	< 2	< 2
Canthaxanthin (mg/100 g)	NA	< 2	< 2	< 2	< 2
Fatty acids (% total FA)					
Myristic – 14:0	0.4	0.4	0.6	0.4	0.6
Palmitic – 16:0	13.9	13.3	15.8	12.8	15.8
Margaric – 17:0	NA	0.1	NA	NA	NA
Heptadecenoic – 17:1	NA	0.2	NA	NA	NA
Hexadecenoic – 16:1 TOTAL	0.1	0.1	0.1	0.1	0.1
Stearic – 18:0	0.7	0.6	0.7	0.6	0.7
Oleic – 18:1(n-9)	0.3	0.2	0.1	0.1	0.1
cis-Vaccenic - 18:1 (n-7)	0.1	0.1	0.1	0.1	0.1
Linoleic – 18:2(n-6)	0.5	0.3	NA	0.1	NA
γ-Linolenic – 18:3 (n-6)	0.2	0.2	0.1	0.2	0.1
α-Linolenic - 18(n-3)	0.4	0.4	0.2	0.4	0.2
Stearidonic -18:4 (n-3)	0.5	0.5	0.3	0.5	0.3
Arachidic – 20:0	0.1	0.1	0.1	0.1	0.1
Homo-γ-linolenic – 20:3(n-6)	0.2	0.2	0.2	0.2	0.2
Arachidonic – 20:4 (n-6)	0.4	0.4	0.4	NA	0.4
Eicosatetraenoic – 20:4(n-3)	0.6	0.6	0.6	0.6	0.6
Eicosapentaenoic (EPA) – 20:5(n-3)	1.1	0.8	0.5	0.8	0.5
Behenic – 22:0	0.1	0.1	0.1	0.1	0.1
Docosapentaenoic (DPA)– 22:5(n-6)	13.4	13.3	13.8	13.4	13.9
Docosapentaenoic (DPA)– 22:5(n-3)	0.3	0.3	0.2	0.2	0.2
Docosahexaenoic (DHA) – 22:6(n-3)	65.1	66.9	64.2	67.7	64.3
Others unidentified	1.6	0.9	1.9	1.6	1.7
trans-Fatty acid	< 0.05[(e)]	< 0.05[(e)]	< 0.05[(e)]	< 0.05[(e)]	< 0.05[(e)]
Sterols					
Total sterol content (mg/kg)	13,375	9,087	NA	NA	9,581
Metals (mg/kg)					
Mercury	< 0.005[(d)]	< 0.005[(d)]	< 0.005[(d)]	< 0.005[(d)]	< 0.005[(d)]
Cadmium	< 0.005[(d)]	< 0.005[(d)]	< 0.005[(d)]	< 0.005[(d)]	< 0.005[(d)]

Parameter (unit)	Batch number				
	0403019	**0413022**	**0418028**	**0419022**	**0419028**
Total arsenic	< 0.01[(d)]	< 0.01[(d)]	< 0.01[(d)]	< 0.01[(d)]	0.01 (±0.003)
Lead	< 0.01[(d)]	< 0.01[(d)]	< 0.01[(d)]	< 0.01[(d)]	< 0.01[(d)]
Copper	< 0.05[(d)]	< 0.05[(d)]	< 0.05[(d)]	< 0.05[(d)]	< 0.05[(d)]
Iron	< 0.2[(d)]	< 0.2[(d)]	0.23 (±0.07)	0.80 (±0.10)	< 0.2[(d)]
Microbiological analysis (CFU/g)					
Aerobic microorganisms 30°C	< 1,000	< 1,000	< 1,000	< 1,000	< 1,000
Moulds	< 10	< 10	< 10	< 10	< 10
Yeast	< 10	< 10	< 10	< 10	< 10
Coliform 30°C	< 1	< 1	< 1	< 1	< 1
Thermotolerant coliforms	< 1	< 1	< 1	< 1	< 1
E. coli β-glucuronidase positive	< 1	Absence/10 g	Absence/10 g	Absence/10 g	Absence/10 g
Coagulase-positive staphylococci	< 10[(b)]	Absence/1 g	Absence/1 g	Absence/1 g	Absence/1 g
Sulfite-reducing anaerobic bacteria	< 1	< 1	< 1	< 1	< 1
Clostridium perfringens	< 1	< 1	< 1	< 1	< 1
Bacillus cereus	< 10	< 10	< 10	< 10	< 10
Salmonella	Absence/25 g	Absence/25 g	Absence/25 g	Absence/25 g	Absence/25 g
Listeria monocytogenes	Absence/25 g	Absence/25 g	Absence/25 g	Absence/25 g	Absence/25 g
Enterobacteria at 30°C	< 10	< 10	< 10	< 10	< 10
PCB and dioxins					
Sum of dioxin and furans (PCDD/Fs TEQ) (pg/g)[(c)]	NA	0.338	0.337	0.172	0.340
PCB (dioxin like – TEQ) (pg/g)[(c)]	NA	0.204	0.204	0.103	0.206
Sum of PCDD/Fs and dl-PCB –TEQ (pg/g)[(c)]	NA	0.543 ± 0.136	0.541 ± 0.135	0.275 ± 0.069	0.546 ± 0.137
PCB (total 6 ndl-PCB) (ng/g)[(c)]	NA	1.97	1.96	0.992	1.98
Polycyclic aromatic hydrocarbons (µg/kg)					
Benzo(a)pyrene	NA	< 0.5	< 0.5	< 0.5	< 0.5
Benzo(a) anthracene	NA	< 0.5	< 0.5	< 0.5	< 0.5
Chrysene	NA	0.8 ± 0.4	< 0.5	< 0.5	< 0.5
Benzo(b)-fluoranthene	NA	0.7 ± 0.5	< 0.5	< 0.5	< 0.5
Sum	NA	1.5 ± 1.1	NA	NA	NA
Process contaminants					
Glycidyl fatty acid esters expressed as glycidol (µg/kg)	NA	Not calculable[(f)]	Not calculable[(f)]	Not calculable[(f)]	22 (± 11)

Parameter (unit)	Batch number				
	0403019	0413022	0418028	0419022	0419028
3-MCPD total (free and fatty acid esters), expressed as 3-MCPD (μg/kg)	NA	< 100[(d)]	< 100[(d)]	< 100[(d)]	150 (± 70)

LOD: limit of detection; NA: not analysed; ND: not detected; LOQ: limit of quantification; MCPD: monochloro-propanol-1,2-diol; PCB: polychlorobiphenyl; dl-PCB: dioxin-like PCB; ndl-PCB: non dioxin-like PCB; PCDD/Fs: polychlorinated dibenzo-*p*-dioxins and dibenzofurans; TEQ: toxicological equivalency.
(a): The applicant provided an additional analysis on total nitrogen in 5 independent batches of the NF which contained proteins below LOQ (0.25%).
(b): A different method was used to analyse this batch as compared to the other batches.
(c): Upper bound results. Upper bound means levels below LOQ (for each congener) are set equal to the LOQ.
(d): LOQ.
(e): LOD.
(f): Glycidyl fatty acid esters were calculated from two parameters 'total 3-MCPD and glycidyl fatty acid esters' and '3-MCPD total'. Since both 'total 3-MCPD and glycidyl fatty acid esters' and '3-MCPD total' were below LOQ (100 μg/kg) it was not possible to calculate 'glycidyl fatty acid esters'.

3.4.1. Stability

In the application initially submitted the applicant did not provide any data on the stability of the NF.

Upon EFSA's request for information, the applicant indicated that the NF is to be stored at a temperature at or below –15°C (frozen conditions), away from light, heat and oxygen.

Upon EFSA's request for information, the applicant provided stability studies on four batches of the NF under the proposed storage conditions (–15°C) as well as at 4°C, 25°C/60% RH and 40°C/75% RH. The content of DHA and markers of oxidation (peroxide value, *p*-anisidine value, TOTOX value and acid value) were analysed regularly during the studies. The markers of oxidation tested were within the limits set in the specifications (peroxide value < 5.0 meqO_2/kg and acid value < 0.5 mg KOH/g) in the NF batches tested at –15°C and 25°C up to 55 weeks (n = 2) and up to 2 years (n = 2), as well as in the NF batches (n = 2) tested at 4°C up to 45 weeks and (n = 3) at 40°C up to 24 weeks. The DHA content remained above 55% (w/w) in all NF batches tested up to the end of the duration of these studies. The NF samples were also tested for *p*-anisidine value and TOTOX value, which remained within the values indicated by the applicant (*p*-anisidine value < 20 and TOTOX value < 26) up to the end of the stability tests. The Panel notes that the *p*-anisidine value, which indicates the oxidative stability of the NF, was below 10 throughout the stability tests.

Based on the stability studies provided, the applicant proposed a shelf life for the NF of 2 years from the date of manufacture, to be stored at a temperature at or below –15°C (frozen conditions), away from light, heat and oxygen.

The Panel considers that the data provided sufficient information with respect to the stability of the NF during the proposed shelf life of 2 years.

3.5. Specifications

As the NF '*Schizochytrium* sp. oil' is already authorised on the EU market, specifications for this NF are currently presented in the Union list. The Panel verified whether the NF under assessment complies with these specifications and subsequently assessed the need to define further specifications for the NF under assessment.

Current specifications of the NF (Union list)

Parameters and corresponding values of the current specifications for '*Schizochytrium* sp. oil' are reported in Table 2. It is noted that the NF currently authorised is not strain specific. Furthermore, the microalgae that is the source of the NF under assessment is currently used as a source for the authorised NF '*Schizochytrium* sp. oil' (see Section 3.2). Therefore, the NF under assessment shall comply with the specifications currently defined for '*Schizochytrium* sp. oil'. A comparison with the data from the batch-to-batch analysis of the present NF is presented in Table 2.

According to the data submitted in the present dossier, the NF produced by the applicant complies with the current specifications for '*Schizochytrium* sp. oil'.

The maximum values observed in the batch-to-batch analysis for the acid value, peroxide value, moisture and volatiles, unsaponifiable and *trans*-FAs are below the limits defined in the specifications (see Table 2). The results of the storage stability studies indicated that the specifications are expected to be met when the NF is stored under the proposed conditions of storage (see Section 3.4.1).

The NF under assessment complies with the specifications for '*Schizochytrium* sp. oil' that is already authorised on the EU market.

Table 2: Specifications of the NF (as currently reported in the Union list) and comparison with analytical content of the NF under assessment

Parameter	Specification for *Schizochytrium* sp. oil (union list)	NF under assessment (based on batch to batch analysis)	Method of analysis
Acid value (mg KOH/g)	≤ 0.5	0.5 (max)	Not reported on the UL
Peroxide value (PV) (meq/kg)	≤ 5.0	2.1 (max)	Not reported on the UL
Moisture and volatile (%)	≤ 0.05	< 0.1 (LOD)	Not reported on the UL
Unsaponifiables (%)	≤ 4.5	1.5 (max)	Not reported on the UL
Trans-fatty acids (%)	≤ 1.0	< 0.05[(a)]	Not reported on the UL
DHA content (%)	≥ 32.0	64.2–67.7[(a)]	Not reported on the UL

DHA: docosahexaenoic acid; LOD: Limit of detection; UL: Union list.
(a): % of fatty acids.

Discussion on additional specifications for the uses assessed in the present dossier

The applicant used a strain of *Schizochytrium* sp. (strain FCC-3204) in the production of the NF and this strain impacts on the FA profile of the NF. Notably, concentrations of DHA range between 64.2% and 67.7% of the FA content, while the minimum required by the specifications is 32% (w/w). Therefore, the applicant proposed to amend the current specifications of the Union list to consider this particularity of the NF. The Panel considers that amending the specification to modify the minimum concentrations of DHA (i.e. > 55% instead of 32%) is not necessary from a safety point of view.

The applicant proposed to add the parameter 'free fatty acids' (≤ 0.3%). The Panel notes that the parameter 'acid value', which is included in the currently authorised specifications, correlates with the presence of free FAs. Thus, the Panel considers that it is not necessary to amend the currently authorised specifications of the NF in this respect.

The Panel notes that the *p*-anisidine value, which allows monitoring the secondary oxidation of oils, has not been considered so far on the Union list for *Schizochytrium* oils. However, secondary oxidation products (such as α,β-unsaturated carbonyl compounds, malonaldehyde) may be of safety concern (Vieira et al., 2017; Kanner, 2007). Therefore, the Panel proposes to add the *p*-anisidine value in the specifications for *Schizochytrium* sp. oils. Considering the European Pharmacopoeia values defined for cod liver and salmon oils (2015) and the compositional data, a maximum limit of 10 could be used for the *p*-anisidine value in *Schizochytrium* oils.

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

3.6.1. History of use of the source

The source of the NF is a microalgae belonging to the genus *Schizochytrium* (see full description in Section 3.2). This genus has been used as a source of DHA-rich oils since 2003, year of the first authorisation of the NF DHA-rich oil from *Schizochytrium* sp. (Commission Decision 2003/427/EC[6]). The first assessment of DHA-rich oil from *Schizochytrium* sp. involved the strain ATCC 2088 (United Kingdom, 2002). However, several other strains have been recognised as valid sources for this NF since 2003.

[6] Commission Decision 2003/427/EC: Commission Decision of 5 June 2003 authorising the placing on the market of oil rich in DHA (docosahexaenoic acid) from the microalgae *Schizochytrium* sp. as a novel food ingredient under Regulation (EC) No 258/97 of the European Parliament and of the Council; OJ L 144, 16.6.2003, p. 13–14.

Following two substantial equivalence assessments (FSAI, 2014 and Anses, 2018), two other strains (FCC-1324 and FCC-3204, respectively) were recognised as valid sources to produce DHA-rich oils equivalent to the original NF. On the current Union list, the DHA-rich oils produced from these strains are commonly referred to as '*Schizochytrium* sp. oil' under Regulation (EU) 2014/463[7].

3.6.2. History of use of the NF

The application under assessment is an extension of use for the first authorised DHA-rich oil, referred to as generic *Schizochytrium* sp. oil. This NF has been authorised since 2003 by means of Commission Decision 2003/427/EC[6]. It has been subject to several extensions of use which were reported in Commission Decision 2009/778/EC[8], Commission Implementing Decision 2014/463/EU[9] and Commission Implementing Regulation 2019/109[10]. The NF *Schizochytrium* sp. oil is currently authorized as a food supplement (250 mg DHA/day for the general population; 450 mg DHA/day for pregnant and lactating women) and as a food ingredient in a wide range of food categories.

A food supplement with the maximum daily intake of 3,000 mg DHA and EPA combined is authorised but is strictly limited to the '*Schizochitrium* sp. oil rich in DHA and EPA'.

3.6.3. Safety assessment for DHA

Upon request of the European Commission, the safety of omega-3 long-chain PUFA (DHA, EPA and DPA), consumed either individually or in combination, was assessed by the NDA Panel in 2012 (EFSA NDA Panel, 2012). The Panel concluded that available data were insufficient to establish a tolerable upper intake level (UL) for EPA, DHA and DPA (individually or combined) for any population group. In the absence of a UL, the Panel provided advice on a daily supplemental intake of n-3 LCPUFAs (i.e. intake of n-3 LCPUFAs taken as food supplement, either individually or combined) which does not give rise to concerns about adverse health effects.

In 2012, based on the available evidence, the Panel concluded that 'supplemental intakes of DHA alone up to about 1 g/day do not raise safety concerns for the general population'. The safety assessment was based on few studies conducted with supplements containing DHA (typically isolated from microalgae). Regarding the combination of EPA and DHA, the Panel concluded 'supplemental intakes of EPA and DHA combined at doses up to 5 g/day do not raise safety concerns for the adult population'. In most human intervention studies considered, fish oils were used as a source of combined EPA and DHA (EFSA NDA Panel, 2012).

3.7. Proposed uses and use levels and anticipated intake

3.7.1. Target population

Upon EFSA's request for clarifications, the applicant indicated that the target population is adults (i.e. individuals aged 18 years and above), excluding pregnant and lactating women.

3.7.2. Proposed uses and use levels

The applicant intends to increase the use level of the NF as a food supplement, from 250 mg DHA/ day (currently authorised for the general population, excluding pregnant and lactating women) to 3 g DHA/day for adults, excluding pregnant and lactating women.

[7] Regulation (EU) 2014/463: Commission Implementing Decision of 14 July 2014 on authorising the placing on the market of oil from the micro-algae *Schizochytrium* sp. as a novel food ingredient under Regulation (EC) No 258/97 of the European Parliament and of the Council and repealing Decisions 2003/427/EC and 2009/778/EC. OJ L 209, 16.7.2014, p. 55–58.

[8] Commission Decision 2009/778/EC: Commission Decision of 22 October 2009 concerning the extension of uses of algal oil from the microalgae Schizochytrium sp. as a novel food ingredient under Regulation (EC) No 258/97 of the European Parliament and of the Council; OJ L 278, 23.10.2009, p. 56–57.

[9] Commission Implementing Decision 2014/463/EU: Commission Implementing Decision of 14 July 2014 on authorising the placing on the market of oil from the micro-algae Schizochytrium sp. as a novel food ingredient under Regulation (EC) No 258/97 of the European Parliament and of the Council and repealing Decisions 2003/427/EC and 2009/778/EC; OJ L 209, 16.7.2014, p. 55–58.

[10] Commission Implementing Regulation (EU) 2019/109 of 24 January 2019 authorising an extension of use of Schizochytrium sp. oil as a novel food under Regulation (EU) 2015/2283 of the European Parliament and of the Council, and amending Commission Implementing Regulation (EU) 2017/2470; OJ L 23, 25.1.2019, p. 7–10.

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

The applicant did not submit specific ADME data for the NF. Digestion, absorption and metabolism of DHA has been extensively documented in the EFSA Scientific Opinion on Tolerable Upper Intake Level of EPA, DHA and DPA (EFSA NDA Panel, 2012).

3.9. Nutritional information

The nutritional content of the NF is provided by the batch-to-batch analysis. The NF mainly consists of fats in the form of triglycerides. *trans*-FAs were not detected, and based on the acid value, free FAs are not expected to be of concern. The FA profile indicates that DHA is the predominant compound in the NF.

The analysis of the composition shows the presence of other nutrients such as sodium, vitamin E, β-carotene. However, given that the proposed maximum daily amount of the NF (3 g DHA which corresponds to 5.5 g of NF), the presence of those nutrients is not expected to be of health concern.

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

3.10. Toxicological information

3.10.1. Qualified presumption of safety (QPS)

The available evidence indicates that the source organism (*Schizochytrium* sp., strain FCC-3204) belongs to the species *S. limacinum*. In 2020, *S. limacinum* 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. In the opinion of the BIOHAZ Panel, *S. limacinum* was identified as a synonym of *Aurantiochytrium limacinum.* 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 *S. limacinum*. The BIOHAZ Panel concluded that *S. limacinum* is recommended for the QPS list with the qualification 'for production purposes only' (EFSA BIOHAZ Panel, 2020).

3.10.2. Absence of marine biotoxins

The absence of marine biotoxins was demonstrated in the assessment of the composition of the NF (see Section 3.4). The Panel investigated whether the LOQs used in the reported analysis of the common marine biotoxins were sufficiently low compared to the acute reference dose (ARfD) of the respective biotoxins (EFSA CONTAM Panel, 2009). It was found that the theoretical intakes resulting from the occurrence of marine biotoxins at their respective LOQs remained well below the respective ARfD of the corresponding biotoxins. It is concluded that the reported LOQs are sufficiently low to ensure consumer safety.

3.10.3. Toxicity of DHA-oils derived from *Schizochytrium* sp.

No toxicity studies that were conducted with the NF under assessment (DHA-rich oil produced from strain FCC-3204 of *Schizochytrium* sp.) have been provided by the applicant.

However, the toxicity of DHA-rich algal oils produced from different strains of *Schizochytrium* sp. has been extensively investigated over the last decades. Several guideline compliant studies, including bacterial reverse mutation tests, *in vitro* chromosomal aberration tests, *in vivo* mammalian cell micronucleus tests, subchronic toxicity studies with rats, and developmental and reproductive toxicity studies with rats, were performed with various forms of DHA algal oils from *Schizochytrium* sp. Most of these studies were assessed and used to conclude on the safety of NFs evaluated in former authorisation frameworks. Notably two studies performed with DHA-oil produced from strain ATCC PTA-9695 (Fedorova-Dahms et al., 2011 and an unpublished study) were performed to support the authorisation of the NF *Schizochytrium* sp. (ATCC PTA-9695) in infant and follow-on formula. These studies have been assessed by the UK competent authority in 2014 (United Kingdom, 2014). Similarly, two other studies performed with DHA-oil produced from strain T18 (Schmitt et al., 2012a,b) have also been considered by the UK competent authority in support of the authorisation of the NF *Schizochytrium* sp. (T18) in infant and follow-on formula in 2017 (United Kingdom, 2017). In addition,

two other studies (Lewis et al., 2016; Falk et al., 2017), performed with DHA-oils from unspecified strains of *Schizochytrium* sp., have been considered in the assessment carried out by Anses (2018).

In all previous assessments, the competent authorities concluded that there were no concerns with regard to genotoxicity and subchronic toxicity of the tested materials. Further studies found in the literature indicate the same outcome for a diversity of DHA-oils produced from other strains of *Schizochytrium* sp. which have a longer history of use (Hammond et al., 2001a, 2001b, 2002; Blum et al., 2007; Kroes et al., 2003; Abril et al., 2003).

3.10.4. Summary

Even though toxicological tests were not conducted with the NF that is assessed in the present opinion, the Panel considers that, given the results on toxicity in studies performed with various forms of DHA-rich oils derived from *Schizochytrium* sp., given the QPS status of the source of the NF, and considering data on the production process and on the composition of the NF and the absence of viable cells, there are no concerns with regard to toxicity of the NF.

3.10.5. Human data

Upon EFSA's request, the applicant provided human studies to support the safety of DHA supplementation for the extension of use of the NF to 3 g DHA/day.

For the assessment of this NF application, the Panel referred to the 2012 EFSA opinion, which concluded that supplemental intakes of DHA alone up to about 1 g/day do not raise safety concerns for the general population. That opinion also concluded that supplemental intakes of EPA and DHA combined at doses up to 5 g/day do not raise safety concerns for the general population. It was noted that in the majority of the human studies considered, fish oils, which also contained DPA in generally unknown (but relatively low) amounts, were the source of EPA and DHA. A typical fish oil supplement provides EPA and DHA in a ratio of 3:2, but 2:1 and higher ratios are also commonly found in fish oils (Harris, 2004; NIH, 2019).

The Panel notes that the EPA:DHA ratio of the NF ranges from 0.008:1 to 0.017:1 based on the batch analyses and hence deviates significantly from the EPA:DHA ratio reported for fish oils. In this context, the applicant claims a conversion factor of DHA into EPA of 3–14% based on studies showing an increase of EPA in serum (Conquer and Holub, 1997, 1998) and in serum and red blood cells (RBC) following ingestion of DHA supplements (Schuchardt et al., 2016). However, the results from a recent study using compound-specific isotope analysis showed that the increase in plasma EPA following DHA supplementation in humans does not occur via retroconversion, but instead from a slowed metabolism and/or accumulation of plasma EPA (Metherel et al., 2019).

The Panel notes that the data gaps and safety concerns identified in the 2012 opinion for supplementation of DHA (alone) were: bleeding complications, bleeding time, glucose homeostasis, LDL-cholesterol and immune function. For the endpoints 'platelet function' and 'lipid peroxidation', the Panel could identify a dose of up to 4–5 g/day of DHA alone which was not considered to have adverse health effects.

Eight clinical trials provided by the applicant were carried out with supplementation of DHA at doses equal or above 3 g/day. Results from several of these clinical trials have been published in different publications reporting on various parameters or outcomes. The Panel notes that no conclusions can be drawn from the study by Rontoyanni et al. (2012) which investigated the effect of single consumption of a meal enriched with DHA.

Details of these clinical trials are summarised in Table 3. The Panel notes that only one clinical trial was conducted with DHA oil from the microalgae *Schizochytrium* sp. The clinical trials reported on effects of supplemental intake of DHA on blood lipid parameters, glucose homeostasis, immune function and/or adverse events. None of these studies addressed possible adverse effects of DHA supplementation on bleeding time or bleeding complications.

Blood lipids

Four clinical trials reported on LDL-cholesterol or other blood lipid parameters in relation to DHA supplementation (≥ 3 g/day) (Kelley et al.*, 2007, 2008; Hughbanks-Wheaton et al., 2014; Domingo et al., 2018a; Klingel et al., 2019).*

Supplementation with 3 g/day of DHA in a trial in hyperlipidemic men showed a statistically significant difference in effect on LDL cholesterol (15% higher in the DHA group – Kelley et al., 2007) and on fasting remnant like particle cholesterol (21% lower in the DHA group – Kelley et al., 2008)

during a 90 day intervention. In a study in HIV-infected men supplemented with 4 g/day of DHA for 48 weeks (Domingo et al., 2018a),no statistically significant differences in blood parameter changes over time were observed between the DHA and the control groups, except for a reduction in triglyceride levels which were more pronounced in the DHA group. In two trials, there were limited and no statistically significant differences in changes in LDL-, HDL- or total cholesterol levels between the group supplemented with DHA and the control group (Hughbanks-Wheaton et al., 2014; Klingel et al., 2019). The Panel notes that in the trial by Hughbanks-Wheaton et al. (2014), only 30% of the study population are above 18 years and correspond to the target population of the NF (i.e. adults).

The Panel notes that the trials tested DHA oils other than the NF, that only one trial was carried out in healthy normolipidemic women and men (Klingel et al., 2019), while the remaining three trials (Kelley et al., 2007, 2008; Hughbanks-Wheaton et al., 2014; Domingo et al., 2018a) were conducted in populations not or only partially corresponding to the target population for the NF (i.e. adults).

The Panel considers that based on the studies provided no conclusions can be drawn on the safety of the NF at the proposed use of 3 g DHA/day on effects on LDL cholesterol or other blood lipids in the general adult population.

Glucose homeostasis

Four clinical trials reported on parameters of glucose homeostasis in relation to DHA supplementation (≥ 3 g/day) (Kelley et al., 2012; Hughbanks-Wheaton et al., 2014; Domingo et al., 2018a; Klingel et al., 2019).

In the trial by Domingo et al. (2018a), fasting glucose, insulin, C-peptide, homeostasis model assessment of insulin resistance (HOMA-IR) and glycosylated haemoglobin levels were not statistically significantly different (as compared to baseline or between the DHA and control groups) throughout the study. The trial by Hughbanks-Wheaton et al. (2014) reported no statistically significant differences in changes from baseline in fasting blood glucose concentrations between the group supplemented with DHA and the control group. In the trial by Klingel et al. (2019), the effect of DHA supplementation on fasting blood glucose concentrations was not statistically significantly different as compared to control. In the trial by Kelley et al. (2012), DHA supplementation statistically significantly increased fasting plasma glucose concentration as compared to baseline, but did not significantly alter other indices of insulin resistance (fasting or postprandial insulin concentrations, postprandial glucose concentrations, HOMA-IR). Changes in the above glucose and insulin parameters were not statistically significantly different between the DHA and control groups.

The Panel notes that the studies provided do not show any adverse effects of DHA supplementation of 3 g/day on the investigated markers of glucose homeostasis. However, the Panel notes that measurements of blood glucose alone (as in the trials by Klingel et al., 2019 and Hughbanks-Wheaton et al., 2014) are not sufficient to draw overall conclusions on possible effects of DHA supplementation on glucose homeostasis. The Panel also notes that glucose and insulin measurements were not the primary outcomes in the trial by Kelley et al. (2012). Lastly, the Panel notes the limitations of these trials regarding the study populations (see Table 3) that did not correspond or partially corresponded to the target population of the NF (i.e. adults).

The Panel considers that based on the evidence provided no conclusions can be drawn on the safety of the NF at the proposed use of 3 g DHA/day on possible effects on glucose homeostasis in the general adult population.

Immune function

Three clinical trials reported on markers of inflammation in relation to DHA supplementation (≥ 3 g/day) (Kelley et al., 2009; Domingo et al., 2018b; Pastor et al., 2019).

The trial by Pastor et al. (2019) was conducted in patients with cystic fibrosis. The Panel considers that no conclusions can be drawn from this study on the effect of DHA supplementation on markers of inflammation.

In a trial in hypertriglyceridemic men (Kelley et al., 2009), a 3 g/day DHA supplementation during a 90 days intervention showed statistically significant changes over time in the DHA group (showing decreases in circulating neutrophils (by 10.5%), white blood cells (by 6%), C-reactive protein (by 15%), interleukin-6 (by 23%) and granulocyte monocyte-colony stimulating factor (by 21%) and an increase (by 7%) in the concentration of anti-inflammatory matrix metalloproteinase-2. However, no statistically significant differences in changes between the DHA group and the control group were reported. In the trial by Domingo et al. (2018b) in HIV patients, there were no statistically significant

differences in changes in the inflammatory parameters investigated between the DHA and the control groups (except for monocyte chemoattractant-protein 1).

The Panel notes that the studies provided do not indicate adverse effects of DHA supplementation of 3 g/day on the investigated markers of inflammation. However, the Panel notes the limitations of these studies regarding the study populations that did not correspond or partially corresponded to the target population of the NF and the limited data on immune functionality. The Panel considers that based on the evidence provided no conclusions can be drawn on the safety of the NF at the proposed use of 3 g DHA/day on possible effects on immune function in the general adult population.

Adverse events

Six clinical trials reported on adverse events in relation to DHA supplementation (≥ 3 g/day) (Kelley et al., 2012; Hoffman et al., 2014; Hughbanks-Wheaton et al., 2014; Oliver et al., 2016; Domingo et al., 2018a; Lee et al., 2019; Metherel et al., 2019).

In the trial reported by Hoffman et al. (2014) and Hughbanks-Wheaton et al. (2014), 51 participants (out of the 78 enrolled) had no related or possibly related treatment emergent adverse events (TEAEs). Twenty-seven participants reported 42 related or possibly related TEAEs (22 in the DHA group and 20 in the control group). Gastrointestinal (GI) events were the main reported events (n = 6 in DHA and n = 4 in the control group). One participant in the DHA group had recurrent GI symptoms such as diarrhoea, flatulence and eructation. Only one blood chemistry TEAE event was observed in the DHA group (elevated serum glutamate pyruvate transaminase), whereas none was observed in the control group. No severe TEAEs requiring hospitalization were reported during the 4-year duration of this trial. In the trial by Kelley et al. (2012), two subjects in the DHA group did not complete the study owing to GI symptoms (feeling of gas/bloating). In the study by Oliver et al. (2016), side effects of supplementation with DHA oil, obtained from microalga *Schizochytrium* sp., were reported by participants (i.e. transient GI distress). These effects were reported only once over the duration of the study. In the trial by Domingo et al. (2018a), the occurrence of TEAEs was not more frequent in the DHA group than in the control group. GI disorders such as fishy smell belching, nausea, and bloating were most common adverse events. They did not lead to treatment discontinuation except for three participants in the DHA group and one participant in the control group. Laboratory parameters including transaminases did not show significant variation in either arm. In the study by Metherel et al. (2019), none of the participants reported any adverse events of the intervention with either DHA or EPA. In the study by Lee et al. (2019), DHA and olive oil (control) evoked similar responses in resting blood pressure, but only DHA increased peripheral vasoconstrictor outflow. In this study, none of the participants reported any adverse effects associated with the interventions.

The Panel notes that the studies provided do not indicate any severe adverse events associated with DHA supplementation up to 6 g/day over several weeks up to 1 year and in one study up to 4 years. Reported adverse events comprise GI effects such as bloating, nausea, unpleasant breath or diarrhoea. The clinical relevance of the elevated peripheral vasoconstrictor outflow observed in one study is unclear.

Overall considerations

In summary, the Panel notes that the study populations in several studies did not correspond to the target population (Kelley et al., 2007, 2008, 2009, 2012; Hoffman et al., 2014; Hughbanks-Wheaton et al., 2014; Domingo et al., 2018a,b), that only one study was carried out with DHA-rich oil from *Schizochytrium* sp. (Oliver et al., 2016) and that only two studies reported on blood chemistry-related TEAEs (Hughbanks-Wheaton et al., 2014; Domingo et al., 2018a). The Panel notes that the parameters of interest for the assessment of this NF were not primary outcomes in the studies provided (except for Kelley et al., 2007) and therefore these studies were not originally designed and powered for the purpose of drawing conclusions on these parameters. No new studies were provided with respect to possible adverse effects of DHA supplementation at the proposed use of the NF on bleeding time or bleeding complications.

Overall, the Panel considers that the available evidence from the studies provided by the applicant is not sufficient to overcome concerns expressed in the 2012 opinion regarding possible adverse effects of DHA supplementation at 3 g/day on LDL-cholesterol concentrations, glucose homeostasis, immune function or bleeding complications. Thus, the Panel considers that the data provided by the applicant are not sufficient to conclude on the safety of the NF at the proposed uses (3 g DHA/day) in adults.

Table 3: Summary of the human intervention studies provided by the applicant which investigated doses equal or above 3 g DHA/day

Authors	Design and primary outcome	Population	Supplementation	Parameters relevant for the safety assessment
Domingo et al. (2018a, b)	Randomised, double-blinded, controlled clinical trial Primary outcome: percentage of change in TG levels at 4 weeks	N = 84 HIV-infected patients, under retroviral therapy, with TG levels between 2.26 and 5.65 mmol/L Sub-study group for markers of inflammation parameters (n = 39; DHA n = 18 and control n = 21)	DHA oil produced by enzymatic synthesis Group 1 (n = 41): 4 g/day of DHA Group 2 (n = 43): control (olive oil) Length of supplementation: 48 weeks	Domingo et al., (2018a) – Blood lipids – Glucose homeostasis – Adverse effects Domingo et al. (2018b) – Markers of inflammation
Hughbanks-Wheaton et al. (2014), Hoffman et al. (2014)	Randomised, double-blinded, controlled clinical trial Primary outcome: rate of loss of cone ERG (electroretinography) function	n = 78 recruited n = 60 mITT that completed ≥ 1 year Males (age range: 7–31 years) with x-linked retinitis pigmentosa	DHA oil from microalga *Crypthecodinium cohinii* Group 1: 30 mg/kg bw per day DHA, corresponding to 600 to 3,600 mg/day of DHA (n = 33 of which n = 12 aged ≥ 18 years old) Group 2: control (corn soy oil) (n = 27 of which n = 7 aged ≥ 18 years old) Length of supplementation: 4 years	Hughbanks-Wheaton et al. (2014) – Blood lipids – Glucose homeostasis – Adverse effects Hoffman et al. (2014) – Adverse effects
Kelley et al. (2007, 2008, 2009, 2012)	Randomised, double-blinded, controlled clinical trial Primary outcomes: TG and lipoprotein particles levels	n = 40 entered the study n = 34 (completed the study) Hypertriglyceridemic men, with the following metabolic parameters: CRP between 1 and 10 mg/L, fasting serum TG concentrations of 150–400 mg/dL, total cholesterol < 300 mg/dL, LDL-cholesterol < 220 mg/dL and BMI between 22 and 35 kg/m^2	DHA oil from microalga *Crypthecodinium cohinii* Group 1 (n = 17): 3 g/day of DHA Group 2 (n = 17): control (olive oil) Length of supplementation: 90 days	Kelley et al. (2007, 2008) – Blood lipids Kelley et al. (2009) – Markers of inflammation Kelley et al. (2012) – Glucose homeostasis – Adverse effects

Authors	Design and primary outcome	Population	Supplementation	Parameters relevant for the safety assessment
Klingel et al. (2019)	Randomised, double-blinded, controlled clinical trial Primary outcome: omega-3 index	n = 89 Young, healthy normolipidemic men and women (aged 21.6 ± 0.23 years)	Supplemented oils obtained from KD Pharma Group 1 (n = 29): 3 g/day of EPA Group 2 (n = 30): 3 g/day of DHA Group 3 (n = 30): control (olive oil) Length of supplementation: 12 weeks	– Blood lipids – Glucose homeostasis
Lee et al. (2019)	Randomised, double-blinded, controlled clinical trial Primary outcomes: blood pressure, omega-3 index, heart rate, muscle sympathetic nerve activity. Calculation of the sample size was based on blood pressure	n = 86 Healthy young men and women aged 18–30 years	Supplemented oils obtained from KD Pharma Group 1 (n =28): 3 g/day of EPA Group 2 (n = 28): 3 g/day of DHA Group 3 (n = 30): control (olive oil) Length of supplementation: 12 weeks	– Adverse effects
Metherel et al. (2019)	Randomised, double-blinded, controlled clinical trial Primary outcome: omega-3 index Calculation of the sample size was based on blood pressure	n = 89 Healthy subjects (45 females, 45 males, aged 21.6 ± 2.2 years, BMI 23.6 ± 3.2 kg/m^2)	Supplemented oils obtained from KD Pharma Group 1 (n = 29): ~ 3 g EPA/day Group 2 (n = 30): ~ 3 g DHA/day Group 3 (n = 30): control (olive oil) Length of supplementation: 12 weeks	– Adverse effects

Authors	Design and primary outcome	Population	Supplementation	Parameters relevant for the safety assessment
Oliver et al. (2016)	Randomised, double-blinded, controlled clinical trial Primary outcome: proportion of DHA in total plasma fatty acids	n = 130 (were randomised) n = 81 (completed the study) American football athletes	DHA oil from microalga *Schizochytrium* sp. Group 1 (n = 21): 2 g/day DHA Group 2 (n = 19): 4 g/day DHA Group 3 (n = 22): 6 g/day DHA Group 4 (n = 19): control (corn oil) Length of supplementation: 189 days	– Adverse effects
Pastor et al. (2019)	Randomised, controlled clinical trial Primary outcomes: surrogate markers of inflammation	n = 50 Patients with cystic fibrosis	DHA seaweed oil Group 1: 50 mg DHA/kg bw per day, corresponding up to 3 g of DHA/day Group 2: placebo Length of supplementation: 12 months	– Markers of inflammation

bw: body weight; BMI: body mass index; LDL: low-density-lipoprotein; mITT: modified intention to treat; TG: triglycerides.

3.11. Allergenicity

Upon EFSA's request for information, the applicant provided a new analysis of 5 batches of the NF, which indicated that proteins were below the LOQ (0.25%). The Panel considers that the NF is unlikely to trigger adverse allergic reactions in the general population or subgroups thereof under the proposed conditions of use.

4. Discussion

The NF, which is the subject of the application, is a DHA-rich oil derived from *Schizochytrium* sp. (FCC-3204). The available evidence indicates that the source organism (*Schizochytrium* sp., strain FCC-3204) belongs to the species *S. limacinum*. The source organism which is assessed in this application is *S. limacinum* (FCC-3204) and not the generic *Schizochytrium* sp. The Panel considers that the information provided on the composition of the NF is sufficient and does not raise safety concerns.

In 2020, *S. limacinum* was assessed by the EFSA BIOHAZ Panel and attributed the QPS status with the qualification 'for production purposes', which implies the absence of viable *Schizochytrium* cells in the final product. Data provided by the applicant demonstrated the absence of the viable cells in the NF. The Panel considers that the production process is sufficiently described and does not raise safety concerns.

The applicant intends to increase the use level of the NF as a food supplement, from 250 mg DHA/day (currently authorised for the general population, excluding pregnant and lactating women) to 3 g DHA/day for adults, excluding pregnant and lactating women.

Toxicological tests with the NF were not performed. However, based on the available toxicological data on various forms of DHA-oils derived from *Schizochytrium* sp., the QPS status of the source of the NF, the production process, the composition of the NF and the absence of viable cells in the NF, the Panel considers that there are no concerns with regard to toxicity of the NF.

The safety concerns identified in the 2012 opinion for supplementation of DHA were bleeding complications, bleeding time, glucose homeostasis, LDL-cholesterol and immune function. The Panel considers that the evidence in human intervention studies provided by the applicant to support the extension of use to 3 g DHA/day in adults (excluding pregnant and lactating women) is not sufficient to overcome these concerns. Therefore, the Panel considers that the data provided by the applicant are not sufficient to conclude on the safety of the NF at the proposed uses (3 g DHA/day) in adults. However, the Panel notes that in the 2012 opinion it was concluded that supplemental intakes of DHA alone up to about 1 g/day did not raise safety concerns for the general population.

5. Conclusions

The Panel concludes that the NF, i.e. *Schizochytrium* sp. oil (produced from the strain FCC-3204 belonging to species *S. limacinum*), is safe for the use in food supplements at the maximum intake level of 1 g DHA/day for the target population proposed by the applicant (adults, excluding pregnant and lactating women).

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 *Schizochytrium* sp. oil as a novel food Ref. Ares(2019) 4415804, dated 10 July 2019.
2) On 10/07/2019, a valid application on *Schizochytrium* sp. oil, which was submitted by Fermentalg, was made available to EFSA by the European Commission through the Commission e-submission portal (NF 2019/1046) and the scientific evaluation procedure was initiated.
3) On 20/12/2019, EFSA requested the applicant to provide additional information to accompany the application and the scientific evaluation was suspended.
4) On 03/09/2020, additional information was provided by the applicant and the scientific evaluation was restarted.
5) During its meeting on 24 November 2020, the NDA Panel, having evaluated the data, adopted a scientific opinion on the safety of oil from *Schizochytrium limacinum* (strain FCC-3204) for use in food supplements as a NF pursuant to Regulation (EU) 2015/2283.

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Abbreviations

ADME	absorption, distribution, metabolism and excretion
AI	adequate intake
ANI	average nucleotide identity
ARfD	acute reference dose
ATCC	American Type Culture Collection
BIOHAZ	Panel on Biohazards
bw	body weight
CCAP	Culture Collection of Algae and Protozoa
CFU	colony forming unit
CONTAM	Panel on Contaminants in the Food Chain
DHA	docosahexaenoic acid
DPA	docosapentaenoic acid
EPA	eicosapentaenoic acid

FA	fatty acids
GI	gastrointestinal
GMO	genetically modified organism
GMP	Good Manufacturing Practice
HACCP	Hazard Analysis Critical Control Points
HDL	high-density lipoprotein
HIV	human immunodeficiency virus
HOMA	homeostasis model assessment
IR	insulin resistance
LC	long chain
LDL	low-density lipoprotein
LOD	limit of detection
LOQ	limit of quantification
MCPD	monochloro-propanol-1,2-diol
ML	maximum level
NDA	Panel on Nutrition, Novel Foods and Food Allergens
NF	novel food
PCB	polychlorobiphenyles
PUFA	polyunsaturated fatty acids
PV	peroxide value
QPS	qualified presumption of safety
RBC	red blood cells
rDNA	ribosomal DNA
RH	relative humidity
SAMS	Scottish Marine Institute
TEAE	treatment emergent adverse events
TEQ	toxicological equivalency
TG	triglyceride
UL	tolerable upper level of intake