Safety of oil from schizochytrium limacinum (strain fcc-3204) for use in infant and follow-on formula as a novel food pursuant to regulation (eu) 2015/2283

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SCIENTIFIC OPINION

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Safety of oil from *Schizochytrium limacinum* (strain FCC-3204) for use in infant and follow-on formula 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 use the NF in infant formulae (IF) and follow-on formulae (FOF). The use level defined by the applicant was derived from Regulation (EU) 2016/127, which states the mandatory addition of DHA to IF and FOF at the level of 20–50 mg/100 kcal. The intake of DHA resulting from the use of the NF in IF and FOF is not expected to pose safety concerns. *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 concludes that the NF is safe under the proposed conditions of use.

Keywords: Novel foods, *Schizochytrium limacinum*, docosahexaenoic acid (DHA), infants and young children, alga, fatty acid, safety

Requestor: European Commission

Question number: EFSA-Q-2019-00187

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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 23 January 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 extend the use of *Schizochytrium* sp. oil to additional food categories, namely, infant and follow-on formulae.

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

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

- In the Scientific Opinion on Dietary Reference Values for fats (EFSA NDA Panel, 2010), the Panel set an adequate intake (AI) of 250 mg for eicosapentaenoic acid (EPA) plus docosahexaenoic acid (DHA) for adults; an AI of 100 mg DHA for infants (> 6 months) and young children < 24 months; and an increase of 100–200 mg preformed DHA in addition to the AI for adults as an adequate supply of n-3 long chain polyunsaturated fatty acids (PUFA) during pregnancy and lactation.
- In the Scientific opinion on nutrient requirements and dietary intakes of infants and young children in the European Union (EFSA NDA Panel, 2013), the Panel concluded on the levels of nutrient and energy intakes that are considered adequate for the majority of infants and young children. In particular, the AI for DHA of 100 mg/day was confirmed for infants and young children between 6 and 24 months and was also applied to infants of 0–6 months, taking into account the concentration of essential fatty acids (FAs; including DHA) in human breast milk. It is noted that EFSA has not set AI for DHA for children after 24 months.
- In the Scientific Opinion on the essential composition of infant and follow-on formulae (EFSA NDA Panel, 2014), the Panel concluded that DHA should be added to IF and FOF due to its structural role in the nervous system and the retina and its involvement in normal brain and visual development. A range for the recommended concentration of DHA in IF and FOF was derived: from 20 mg/100 kcal (4.8 mg/100 kJ), based on the AI of DHA (100 mg/day) and an average energy intake of 500 kcal/day, to 50 mg/100 kcal (12 mg/100 kJ) based on the highest observed DHA concentration in human milk (1% DHA in FAs) and the amount of FA in human milk.

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].

[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.

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 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. (strain FCC-3204)'. 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 PUFA in which DHA represents more than 55%. The NF is proposed to be used as an ingredient in infant formulae (IF) and follow-on formulae (FOF).

3.2. Identity of the NF

The NF under assessment in the present application is an oil rich in docosahexaenoic acid (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*.

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 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

[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

(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 *Schizochytrium 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 *A. 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 Hazard Analysis Critical Control Point (HACCP) system and Good Manufacturing Practice (GMP).

The microalgal biomass is lysed directly via an enzymatic hydrolysis. This lysis involves a food-grade and non-genetically modified organism (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.

The NF produced by the applicant is an oil which may undergo further processing steps (e.g. powdering) to be used as an ingredient of IF and FOF. However, these steps are not carried out by the applicant, but by manufacturers of IF and FOF (see also Section 3.4.1 Stability of the NF under the intended conditions of use). Therefore, the description of the production process ends with the packaging and storing of the NF in its liquid/oily form.

3.4. Compositional data

The NF consists of triglycerides composed of 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.

In order to confirm that the manufacturing process is reproducible and adequate to produce a product with the required characteristics on a commercial scale, the applicant provided analytical information for 5 independent batches of the NF (Table 1). Information was provided on the accreditation of the laboratories that conducted the analyses presented in the application.

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 five independent batches of the NF which reported contents of proteins below the limit of quantification (LOQ) (0.25%).

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).

The composition of the NF which is under assessment can be compared to the composition of the two authorised NFs based on DHA-rich oil from *Schizochytrium* currently and authorised for uses in IF and FOF: '*Schizochytrium* sp. (ATCC PTA-9695) oil' and '*Schizochytrium* sp. (T18) oil'. Compositional data for these two authorised NFs are available in the dossiers previously assessed by national authorities and submitted by DSM (2013) and Mara Renewable (2016). A comparison of the main long-chain (n-3)-PUFA relevant in marine oils (DHA, EPA and DPA) was made. In the present NF, the average DHA content (65% of FA) is higher compared to the two authorised NFs, in which the DHA content ranged between 37% and 44% FA. The average EPA content in the present NF (0.74% FA) is lower compared to the two authorised NFs (6% FA and 1.3% FA). Regarding DPA (n-3) concentration is low in the present NF (0.24% FA), which is also the case in the two authorised NFs (< 1% FA).

In terms of chemical contaminants, the concentrations of metals (< LOQs), 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. Four batches of the NF were tested for the process contaminants glycidyl fatty acid esters (expressed as glycidol) and total 3-monochloro-propanol-1,2-diol (MCPD) (free and fatty acid esters). The Panel notes that for glycidyl fatty acid esters (expressed as glycidol), the maximum level (ML) in IF and FOF (liquid) is set at 6 μg/kg. For 3-MCPD (sum of 3-MCPD and 3-MCPD fatty acid esters, expressed as 3-MCPD), a ML in IF and FOF (liquid) is set at 15 μg/kg. The MLs for these contaminants are expected to be respected in the final product (IF and FOF) considering the maximum concentration of the NF in IF and FOF (63 mg NF/100 mL). 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[(f)]	< 0.5[(f)]	< 0.5[(f)]	0.6	< 0.5[(f)]
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

Parameter (Unit)	Batch number				
	0403019	0413022	0418028	0419022	0419028
Physico-chemical parameters					
***p*-Anisidine value**	6.1	2.0	7.3	1.6	5.6
Moisture and volatiles (%)	< 0.1[(g)]	< 0.1[(g)]	< 0.1[(g)]	< 0.1[(g)]	< 0.1[(g)]
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
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

Parameter (Unit)	Batch number				
	0403019	**0413022**	**0418028**	**0419022**	**0419028**
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[(g)]	< 0.05[(g)]	< 0.05[(g)]	< 0.05[(g)]	< 0.05[(g)]
Sterols					
Total sterol content (mg/kg)	13375	9087	NA	NA	9581
Metals (mg/kg)					
Mercury	< 0.005[(f)]	< 0.005[(f)]	< 0.005[(f)]	< 0.005[(f)]	< 0.005[(f)]
Cadmium	< 0.005[(f)]	< 0.005[(f)]	< 0.005[(f)]	< 0.005[(f)]	< 0.005[(f)]
Total arsenic	< 0.01[(f)]	< 0.01[(f)]	< 0.01[(f)]	< 0.01[(f)]	0.01 (± 0.003)
Lead	< 0.01[(f)]	< 0.01[(f)]	< 0.01[(f)]	< 0.01[(f)]	< 0.01[(f)]
Copper	< 0.05[(f)]	< 0.05[(f)]	< 0.05[(f)]	< 0.05[(f)]	< 0.05[(f)]
Iron	< 0.2[(f)]	< 0.2[(f)]	0.23 (± 0.07)	0.80 (± 0.10)	< 0.2[(f)]
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

Parameter (Unit)	Batch number				
	0403019	0413022	0418028	0419022	0419028
Sum	NA	1.5 ± 1.1	NA	NA	NA
Process contaminants					
Glycidyl fatty acid esters expressed as glycidol (μg/kg)[(d)]	NA	Not calculable[(h)]	Not calculable[(h)]	Not calculable[(h)]	22 (± 11)
3-MCPD total (free and fatty acid esters), expressed as 3-MCPD (μg/kg)[(e)]	NA	< 100[(f)]	< 100[(f)]	< 100[(f)]	150 (± 70)

LOD: limit of detection; MCPD: monochloro-propanol-1,2-diol; NA: not analysed; ND: not detected; LOQ: limit of quantification; 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): It is noted that glycidyl fatty acid esters in infant formulae (IF) should not exceed the maximum level (ML) of 6 μg/kg for liquid IF and FOF while a maximum concentration of 22 μg/kg was found in batch 0419028. However, considering the maximum concentration of the NF in IF (63 mg NF/100 mL), the ML of 6 μg/kg in IF is expected to be respected.

(e): It is noted that the sum of 3-MCPD and 3-MCPD esters, expressed as free 3-MCPD in IF should not exceed the maximum level (ML) of 15 μg/kg for liquid IF and FOF while a maximum concentration of 150 μg/kg (sum expressed as 3-MCPD) was found in batch 0419028. However, considering the maximum concentration of the NF in IF (63 mg NF/100 mL), the ML of 15 μg/kg in IF is expected to be respected.

(f): LOQ.

(g): LOD.

(h): 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

Stability of the NF

The applicant performed two stability tests with three independently produced batches of the NF: one test up to 12 weeks at 25°C and a second test up to 6 months at 40°C. The batches were regularly analysed for peroxide index ($meqO_2$/kg), *p*-anisidine value and DHA content (mg/g).

In the 12-week test performed at 25°C, peroxide index increased with time (from 1 to 7.8–9.5 $meqO_2$/kg) and quickly exceeded the value of 5 $meqO_2$/kg (used in the current specifications; see also Section 3.5). The *p*-anisidine value increased 26-45% (maximum value 8.7 after 12 weeks). Concentration of DHA did not change significantly during storage (from 586-601 to 578-586 mg/g).

In the 6-month test at 40°C, peroxide index increased with time (from 1 to 10.6-17.2 $meqO_2$/kg) and quickly exceeded the value of 5 $meqO_2$/kg (used in the current specifications; see also Section 3.5). The *p*-anisidine value considerably increased with a factor of 6–19 (maximum value 45.5 after 6 months of storage). The concentration of DHA remained unchanged (from 586-601 at t = 0 to 562-579 mg/g at t = 6 months).

The results of both studies indicate that the NF is subject to oxidation when stored at 25°C or 40°C. It is noted that the increase of the oxidation parameters was linear and that the peroxide index was already above the limit defined by the specification (5 $mgeqO_2$/kg) after 6 weeks of storage at 25°C.

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.

EFSA requested the applicant to provide new stability studies covering the recommended storage conditions for the NF. Thus, 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.

Stability of the NF under the intended conditions of use (i.e. when the NF oil is powdered to be incorporated to IF and FOF)

According to the conditions of use proposed by the applicant, the NF is intended to be incorporated in IF and FOF. The applicant indicated that the NF is micro-encapsulated into a powdered form before being incorporated into IF and FOF.

During the assessment, EFSA requested information on the stability of the NF when undergoing processing of powdering into IF and FOF and the storage of the powder. In reply, the applicant provided the results of the content of DHA and markers of oxidation (peroxide value, *p*-anisidine value, TOTOX value and acid value) of three batches of the NF before and after being micro-encapsulated into a powder form. An increase in the oxidative parameters was observed after the NF being encapsulated. However, these parameters remained within the specified values (peroxide value < 5.0 meqO_2/kg; acid value < 0.5 mg KOH/g; *p*-anisidine value < 20; TOTOX value < 26). The DHA content, which remained above 55% (w/w), slightly decreased by 4–5% after the micro-encapsulation.

The Panel notes that the data on oxidative parameters and DHA content relate only to the time point after processing the NF into powder and did not cover a longer time span. However, considering the stability data of the NF at 25°C/60% RH up to 2 years, the Panel expects the NF to be stable under the intended conditions of use (i.e. as a microencapsulated powder and when added to IF and FOF).

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.

It is noted that the safety assessment of the NF for its use in IF and FOF required a detailed analysis of potential contaminants. Most of them are covered by sectoral legislation (e.g. Regulation on infant formulae). For marine biotoxins, however, risk managers may consider amending the specifications for the NF '*Schizochytrium* sp. oil' when authorised in IF and FOF in order to set the limits at the LOQ achieved in the batch-to-batch analysis.

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 (Kanner, 2007; Vieira et al., 2017). 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). Table 3 presents the different entries referring to oils from the genus *Schizochytrium* which are either currently present in the Union list (*Shizochytrium* sp.) or have been assessed by EFSA (*Schizochytrium limacinum* WZU477).

Table 3: Overview of the entries referring to oils from *Schizochytrium* sp. which are either currently present in the Union list or have been assessed by EFSA

Name of the novel food	Year of 1st authorisation	Decisions or EFSA opinion	Remarks
Schizochytrium sp. oil	2003	Decision 2003/427/EC[(a)], 2009/778/EC[(b)], 2014/463/EU[(c)] and 2019/109[(d)]	
Schizochytrium sp. oil rich in DHA and EPA	2012	Authorised by the United Kingdom	–
Schizochytrium sp. (ATCC PTA-9695) oil	2015	Decision 2015/545[(e)]	Authorised use on IF and FOF
Schizochytrium sp. (T18) oil	2017	Authorised by Ireland	Authorised use on IF and FOF
Oil from *Schizochytrium limacinum* (strain WZU477)		EFSA opinion on the safety of oil from *Schizochytrium limacinum* (strain WZU477) for use in infant and follow-on formula as a novel food pursuant to Regulation (EU) 2015/2283	Adopted by the NDA Panel on 31/8/2020 (https://doi.org/10.2903/j.efsa.2020.6242)

DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid; IF: infant formulae; FOF: follow-on formulae.

(a): 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.

(b): 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.

(c): 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.

(d): 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.

(e): Commission Implementing Decision (EU) 2015/545 of 31 March 2015 authorising the placing on the market of oil from the micro-algae *Schizochytrium* sp. (ATCC PTA-9695) as a novel food ingredient under Regulation (EC) No 258/97 of the European Parliament and of the Council; OJ L 90, 2.4.2015, p. 7–10.

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.

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]. It is noted that these authorisations currently do not cover the use of these oils in IF and FOF.

Since 2015, two other strains belonging to the genus *Schizochytrium* were also authorised for the production of DHA-rich oils: strain ATCC PTA-9695 (2015) and strain T18 (2017). These NFs were also authorised for use in IF and FOF.

3.6.2. History of use of the NF

DHA-rich oils from *Schizochytrium* have been on the EU market since 2003. On the Union list, there are currently four different entries referring to *Schizochytrium* DHA-rich oils (Table 3).

[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.

[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.

The NF application under assessment is an extension of use for the oil referred to as generic *Schizochytrium* sp. oil that 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 under assessment (*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 (use levels are expressed in mg of DHA): Milk-based drinks and similar products intended for young children (200 mg/100 g); Processed cereals-based food and baby foods for infants and young children (200 mg/100 g); Food intended to meet the expenditure of intense muscular effort (200 mg/100 g); Food-bearing statements on the absence or reduced presence of gluten (200 mg/100 g); Food for specific medical purposes; Bakery products (breads, rolls and sweet biscuits) (200 mg/100 g); Cereal bars (500 mg/100 g); Breakfast cereals (500 mg/100 g); Cooking fats (360 mg/100 g); Dairy analogues except drinks (200-600 mg/100 g); Dairy products except milk-based drinks (200–600 mg/100 g); Non-alcoholic beverages (including dairy analogue and milk-based drinks) (80 mg/100 mL); Spreadable fats and dressings (600 mg/100 g); Fruit and vegetables puree (100 mg/100 g).

Besides the NF under assessment, other *Schizochytrium* sp. oils are also present on the Union list: *Schizochitrium* sp. oil rich in DHA and EPA; *Schizochytrium* sp. (ATCC PTA-9695) oil and *Schizochytrium* sp. (T18) oil. These NFs were authorised for the same food categories as the generic NF *Schizochytrium* sp. oil reported above, plus some additional uses. In particular, the two strain-specific NFs *Schizochytrium* sp. (ATCC PTA-9695) oil and *Schizochytrium* sp. (T18) oil were authorised for use in IF and FOF. These NFs can be used in accordance with Regulation (EU) 609/2013[11], which was supplemented by Regulation (EU) 2016/127[12].

3.7. Proposed uses and use levels and anticipated intake

3.7.1. Target population

The NF is intended to be added in IF and FOF. Consequently, the target population defined by the applicant is infants and young children.

3.7.2. Proposed uses and use levels

The NF is intended to be added to IF and FOF. The proposed use levels are in accordance with Regulation (EU) No 609/2013 and its supplementing Regulation (EU) 2016/127, which states the mandatory addition of DHA to IF and FOF at levels ranging between 4.8 and 12 mg/100 kJ (eq. 20–50 mg/100 kcal). Considering a standard energy content of maximum 70 kcal/100 mL of IF/FOF defined in Regulation (EU) 2016/127, the DHA level in the reconstituted formula is expected to range between 14 and 35 mg DHA/100 mL. Considering a minimum DHA concentration of 550 mg DHA/g in the NF, the use level for the NF corresponds to 25–63 mg NF/100 mL, to reach the target of 14–35 mg DHA/100 mL.

[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.

[11] 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 Text with EEA relevance; OJ L 181, 29.6.2013, p. 35–56.

[12] Commission Delegated Regulation (EU) 2016/127 of 25 September 2015 supplementing Regulation (EU) No 609/2013 of the European Parliament and of the Council as regards the specific compositional and information requirements for infant formula and follow-on formula and as regards requirements on information relating to infant and young child feeding; OJ L 25, 2.2.2016, p. 1–29.

It should be noted that manufacturers of IF and FOF who may powder the NF and incorporate it into their formulae shall guarantee that the concentration of DHA meets the requirement of the Regulation. This is also the case if other sources of DHA are used in combination with the NF.

3.7.3. Anticipated intake of the NF

As the proposed use levels are in accordance with Regulation (EU) No 609/2013 and its supplementing Regulation (EU) 2016/127, the intake of DHA for infants fed with IF supplemented with the NF at the proposed use level is within the range foreseen by the Regulation.

The Panel assessed the maximum intake of NF resulting from the use of the NF in IF. The conservative scenario where IF is the only food consumed by non-breastfed infants from 0 to 4 months was considered, using the default value of 260 mL/kg body weight (bw) per day for high formula intakes for infants 0–4 months (EFSA Scientific Committee, 2017). Based on the use level defined by the applicant (maximum concentration of NF in IF of 63 mg/100 mL), the high intake of NF resulting from the consumption of IF is estimated to be 163.8 mg NF/kg bw per day.[13] Considering that 55% of the NF is DHA, the estimated high daily intake of DHA from IF is 90 mg/kg bw per day.

Furthermore, two other DHA-rich oils from *Schizochytrium*[14] are currently already authorized for use in IF and FOF, with use levels also in line with Regulation (EU) 2016/127. Consequently, the intended use of IF and FOF currently under assessment for the NF *Schizochytrium* sp. oil (from strain FCC-3204) is not expected to modify the current daily intake of DHA-rich oil for infants and young children. The NF is proposed by the applicant as an alternative source of DHA for IF and FOF.

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 have 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 fat 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 reveals that DHA is the predominant compound. DHA is an essential nutrient for infants and children. The essential role of DHA for the development of the nervous system and the retina in infants and young children has been documented in the EFSA Scientific Opinion on nutrient requirements and dietary intakes of infants and young children in the European Union (EFSA NDA Panel, 2013). When used in accordance with the proposed use level, the NF can enrich the composition of IF and FOF to such extent that the latter fall within the range targeted by the current Regulation (20–50 mg DHA/100 kcal).

The concentration of sterols in the NF ranges between 9,000 and 13,400 mg/kg and corresponds to 0.0057–0.0084 mg/mL in IF and FOF added with 63 mg NF/100 mL. The concentration of sterols in IF and FOF added with the NF is below the concentration of sterols reported in marketed IF and FOF (total animal sterols: 0.017–0.054 mg/mL; total plant sterols: 0.03–0.05 mg/mL reported by Claumarchirant et al., 2015; total sterols: 0.09–0.15 mg/mL reported by Hamdan et al., 2018).

The analysis of the composition shows the presence of other nutrients such as sodium, vitamin E, beta-carotene. However, given that the NF will be incorporated into IF and FOF at a maximum concentration of 63 mg NF/100 mL, the presence of those nutrients in the reconstituted formulae is not expected to be of health concern.

The analysis of the FA profile of the NF shows the presence of other components that might affect the overall ratio of FA in IF and FOF. However, it falls under the responsibility of the manufacturers to guarantee that the overall ratio of FA complies with the current regulations.

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.

[13] The mean body weight of a 0- to 3-month infant is 5 kg and is used as a default body weight for the whole group of infants (EFSA Scientific Committee, 2012).

[14] *Schizochytrium* sp. (ATCC PTA-9695) oil and *Schizochytrium* sp. (T18) (see Section 3.6.2).

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 *A. 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.

3.11. Allergenicity

Upon EFSA's request for information, the applicant provided a new analysis of five 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 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 market the NF as an ingredient for IF and FOF to meet the requirement of Regulation (EU) 2016/127. Consequently, the proposed use levels (defined on DHA basis) are the same as for the other DHA-rich oils from *Schizochytrium* sp. which are currently on the market and authorised for supplementing DHA in IF and FOF. Therefore, the intake of DHA resulting from the proposed use is not expected to modify the current situation as regards the total intake of DHA in infants and young children.

Toxicological tests with the NF were not performed. However, based on the available toxicological data of 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 absence of viable cells in the NF, the Panel considers that there are no concerns with regard to toxicity of the NF.

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 under the proposed conditions of use. The target population is infants and young children.

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)3149182, dated 13 May 2019.
2) On 13/05/2019, a valid application on *Schizochytrium* sp. oil as a novel food, which was submitted by Fermentalg, was made available to EFSA by the European Commission through the Commission e-submission portal (NF 2019/0825) and the scientific evaluation procedure was initiated.
3) On 18/11/2019, EFSA requested the applicant to provide additional information to accompany the application and the scientific evaluation was suspended.
4) On 07/09/2020, 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 *S. limacinum* (strain FCC-3204) for use in infant and follow-on formula 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
FOF	follow-on formula
GMO	genetically modified organism
GMP	Good Manufacturing Practice
HACCP	Hazard Analysis Critical Control Point
IF	infant formula
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	polychlorobiphenyl
PUFA	polyunsaturated fatty acids
PV	peroxide value
QPS	qualified presumption of safety
rDNA	ribosomal DNA
RH	relative humidity
SAMS	Scottish Marine Institute
TG	triglyceride
TEQ	toxicological equivalency