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SCIENTIFIC OPINION

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Safety of oil from *Schizochytrium limacinum* (strain ATCC-20889) for use in infant and follow-on formula as a novel food pursuant to Regulation (EU) 2015/2283

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The declarations of interest of all scientific experts active in EFSA's work are available at https://open.efsa.europa.eu/experts

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 an oil derived from the microalga *Schizochytrium* sp. (strain ATCC-20889) as a novel food (NF) pursuant to Regulation (EU) 2015/2283. The NF is a mixture of triglycerides in which docosahexaenoic acid (DHA) represents 40%–43% of fatty acids. The available evidence indicates that the source organism (strain ATCC-20889) belongs to the species *Schizochytrium limacinum*. The applicant intends to market the NF as an ingredient in infant formulae (IF) and follow-on formulae (FOF). The use levels proposed by the applicant were derived from Regulation (EU) 2016/127, which states the mandatory presence of DHA to IF and FOF at the levels of 20–50 mg/100 kcal. *Schizochytrium 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, the absence of marine biotoxins and viable cells in the NF, the Panel considers that there are no concerns with regard to the toxicity of the NF. The Panel concludes that the NF is safe under the proposed conditions of use.

KEYWORDS

algae, docosahexaenoic acid (DHA), fatty acid, infants and young children, novel foods, safety, *Schizochytrium*

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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, as a novel food for use in a number of foods as listed in Commission Implementing Regulation (EU) 2017/2470 establishing the Union list of novel foods (NF) in accordance with Regulation (EU) 2015/2283.

On 24 May 2023, the company BioPlus Life Sciences submitted a request to the European Commission in accordance with Article 10 of Regulation (EU) 2015/2283 for modification of the conditions of use of *Schizochytrium* sp. oil as a NF.

The application requests to authorise use of *Schizochytrium* sp. oil, produced from *Schizochytrium* sp. ATCC 20889, in additional food categories, namely, infant formula (IF) and follow-on formula (FOF) as defined by Regulation (EU) No 609/2013.

Please note that an application for this NF had been already submitted in 2019 (NF 2019/1213) and evaluated by EFSA (EFSA-Q-2019-00548). In its scientific opinion 'Safety of oil from *Schizochytrium* sp. (strain ATCC-20889) for use in infant and follow-on formula as a novel food pursuant to Regulation (EU) 2015/2283' (EFSA NDA Panel, 2022) EFSA concluded that the safety of the NF *Schizochytrium* sp. oil, produced from the strain ATCC-20889, has not been established. Therefore, the EFSA opinion did not give sufficient grounds to establish that the novel food at the proposed uses and use levels complied with the requirements of Article 7 of Regulation (EU) 2015/2283. Based on the EFSA opinion, the Commission considered that the authorisation of *Schizochytrium* sp. (ATCC-20889) oil as a NF as requested by the applicant was not justified and the procedure was terminated by the Commission in accordance with Article 10(6) of Regulation (EU) 2015/2283 without the update of the Union list.

In accordance with Article 29(l)(a) of Regulation (EC) No 178/2002, the European Commission asks EFSA to provide a scientific opinion on the change of the conditions of use of *Schizochytrium* sp. oil as a NF in accordance with Article 10(3) of Regulation (EU) 2015/2283.

1.2 | Additional information

Three existing evaluations of the EFSA NDA Panel 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 additional 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 milk. It is noted that EFSA has not set an AI for DHA for children older than 24 months.
- In the Scientific Opinion on the essential composition of IF and FOF (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 in the safety assessment of this application (EFSA BIOHAZ Panel, 2020, 2023).

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.[1]

A common and structured format on the presentation of NF applications is described in the EFSA guidance on the preparation and presentation of an NF application (EFSA NDA Panel, 2021). As indicated in this guidance, it is the duty of the

[1]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.

applicant to provide all the available (proprietary, confidential and published) scientific data (including both data in favour and not in favour) that are pertinent to the safety of the NF.

The applicant has submitted a confidential and a non-confidential version of the dossier following the 'Guidance on the preparation and submission of an application for authorisation of a novel food in the context of Regulation (EU) 2015/2283 (Revision 1)' (EFSA NDA Panel, 2021) and the 'Administrative guidance for the preparation of applications on NF pursuant to Article 10 of Regulation (EU) 2015/2283' (EFSA, 2021a).

In accordance with Art. 38 of Regulation (EC) No 178/2002[2] and taking into account the protection of confidential information and of personal data in accordance with Articles 39–39e of the same Regulation, and of the Decision of EFSA's Executive Director laying down practical arrangements concerning transparency and confidentiality,[3] the non-confidential version of the dossier has been published on Open.EFSA.[4]

According to Art. 32c (2) of Regulation (EC) No 178/2002 and to the Decision of EFSA's Executive Director laying down the practical arrangements on pre-submission phase and public consultations,[2] EFSA carried out a public consultation on the non-confidential version of the technical dossier from 4 June 2024 to 25 June 2024; no comments were received.

This NF application includes a request for protection of proprietary data in accordance with Article 26 of Regulation (EU) 2015/2283. The data requested by the applicant to be protected are listed in Appendix A.

2.2 | Methodologies

The assessment follows the methodology set out in the EFSA Guidance on NF applications (EFSA NDA Panel, 2021) and the principles described in the relevant existing guidance documents from the EFSA Scientific Committee. The legal provisions for the assessment of NFs are laid down in Article 11 of Regulation (EU) 2015/2283 and in Article 7 of Commission Implementing Regulation (EU) 2017/2469.

The legal provisions for the assessment of food intended for infants and young children are laid down in Regulation (EU) 609/2013 and in Commission Delegated Regulation (EU) 2016/127.

This assessment concerns only the risks that might be associated with consumption of the NF under the proposed conditions of use and is not an assessment of the efficacy of the NF with regard to any claimed benefit.

3 | ASSESSMENT

3.1 | Introduction

The NF, which is the subject of this application, is an oil that is produced by the microalga *Schizochytrium* sp. (strain ATCC-20889). With reference to article 3 of the NF Regulation (EU) 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 microalgae followed by extraction and refinement of the oil produced by the microalgae. The oil is a mixture of triglycerides in which DHA represents around 40% of total FAs. *Schizochytrium* sp. oil is authorised, in accordance with Regulation (EC) No 258/97, as a NF for use in a number of foods as listed in the Union list of NF (Commission Implementing Regulation (EU) 2017/2470). The NF which is subject to this application is proposed to be used as an ingredient in IF and FOF.

3.2 | Identity of the NF

The NF under assessment in the present application is an oil from the microalga *Schizochytrium* sp. (strain ATCC-20889). The oil is a mixture of triglycerides in which DHA is the predominant FA (40–43%), making up together with docosapentaenoic (DPA; n-6) and palmitic acid between 80% and 85% of total FAs. The applicant indicated that the oil is derived from marine microalgae belonging to the genus *Schizochytrium*. The taxonomic classification of the genus *Schizochytrium* has been subject to discussions in 2007 (Yokohama & Honda, 2007) and the genus *Schizochytrium* can now also be referred to as *Aurantiochytrium*.

The applicant indicated that the strain used to produce the NF is *Schizochytrium* sp. ATCC-20889. This strain has not been developed by the applicant, and it has been obtained from the American Type Culture Collection (ATCC) under the reference number 'ATCC-20889'.

The applicant performed the whole genome sequence (WGS) analysis of the strain ATCC-20889 and compared it with the genome sequence of the strain *Schizochytrium limacinum* ATCC MYA-1381 following the EFSA Statement (EFSA, 2021b). The total length of *S. limacinum* ATCC MYA-1381 and ATCC-20889 genomes were 61,568,109 base pairs (bp) and 61,423,524

[2]Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p.1–48.

[3]Decision available at: https://www.efsa.europa.eu/en/corporate-pubs/transparency-regulation-practical-arrangements.

[4]The non-confidential version of the dossier has been published on Open.EFSA and is available at the following link: https://open.efsa.europa.eu/consultations/a0cTk000002nmNRIAY?search=EFSA-Q-2023-00410.

bp, respectively. The average length of scaffolds was 1,664,003 bp for ATCC MYA-1381 and 1,660,095 bp for ATCC-20889. According to the applicant, the average nucleotide identity (ANI) between the genomes of ATCC-20889 and ATCC MYA-1381 was found to be 99.97%. Following the EFSA Statement (EFSA, 2021b), the applicant also compared the WGS of both strains ATCC-20889 and ATCC MYA-1381 with the publicly available genomes of other strains belonging to the species *S. limacinum* in the National Center for Biotechnology Information (NCBI). ANIs between *Schizochytrium* ATCC-20889 and *A. limacinum* BL10 v1, *A. limacinum* CCAP_4062/1 and *S. limacinum* SR21 were 98.98, 98.92 and 99.99%, respectively. ANIs between *S. limacinum* ATCC MYA-1381 and *A. limacinum* BL10 v1, *A. limacinum* CCAP_4062/1 and *S. limacinum* SR21 were 98.79, 98.91 and 99.99%, respectively. Following EFSA's request to perform phylogenomic analyses according to the EFSA Statement (EFSA, 2021b) with different housekeeping genes, the applicant performed a phylogenetic analysis of 18S rRNA sequences, 28S rRNA sequences and complete ITS region (18S rRNA, ITS1, 5.8S rRNA, ITS2 and 28S rRNA). These analyses showed that *Schizochytrium* ATCC-20889 and *S. limacinum* ATCC MYA-1381 belong to the species *S. limacinum*.

Based on the data provided, the Panel considers that the strain ATCC-20889 is a member of the species *Schizochytrium limacinum*.

3.3 | Production process

According to the information provided, the NF is produced in line to good manufacturing practices (GMP) and hazard analysis critical control points (HACCP) principles.

The unicellular microalgae *Schizochytrium* sp. (strain ATCC-20889) are grown under controlled conditions (time, temperature, pH and aeration) in a sterile liquid culture medium containing the necessary nutrients. The microalgal biomass is separated from the culture medium by using a decanter. The microalgal biomass is subjected to cell disruption via a solvent (ethyl acetate) under defined pressure and temperature conditions. After centrifugation to remove cell debris, the solvent is evaporated to yield crude oil. The crude oil is winterised and centrifuged. The crude oil is refined using standard techniques (neutralisation, bleaching, deodorisation). High oleic sunflower oil is added prior to deodorisation to adjust the DHA content. EU-authorised antioxidants (ascorbyl palmitate and tocopherols) are also added to ensure stability. The NF is finally filtered and packed under nitrogen conditions.

Considering the data provided by the applicant on the absence of viable cells in the NF, the solvent used, the pressure and the temperature applied in the production process (e.g. deodorisation) as well as the filtration step applied, the Panel considers that viable cells are not expected to remain in the NF. Considering the temperature applied during the refining steps, the Panel considers that potential residual of the solvent used to lyse the microalgae is removed.

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

The NF produced by the applicant is an oil which may undergo further processing steps (microencapsulation) 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. Therefore, the description of the production process of the NF ends with the packaging and storing of the NF in its liquid/oily form at –20°C, under nitrogen atmosphere.

3.4 | Compositional data

The NF consists of a mixture of triglycerides, mainly composed of PUFA, in which DHA is the predominant one (40%–43%), making up together with DPA (n-6) and palmitic acid between 80% and 85% of total FAs.

In order to confirm that the manufacturing process is reproducible and adequate to produce a product with the required characteristics, the applicant provided analytical information for five batches of the NF after the addition of sunflower oil and after deodorisation (Table 1). Upon EFSA's request for information, the applicant clarified that the batches of the NF are routinely tested against the specifications before and after the addition of the sunflower oil. In this respect, the applicant provided the results of the analysis of five batches of the NF prior to the addition of sunflower oil and prior to deodorisation (Table 2). Table 1 includes the results of the microbiological analysis of the NF.

The applicant tested five batches of the NF for organic volatile impurities (sum of ethyl acetate and n-hexane), which were below the limit of quantification (LOQ = 5.5 mg/kg).

In terms of chemical contaminants, the concentrations of heavy metals, polychlorobiphenyls, dioxins, polycyclic aromatic hydrocarbons and pesticides in this batch-to-batch analysis were below LOQs and within the EU limits established in the respective regulations and do not raise safety concerns. The Panel notes the presence of erucic acid in the NF, which is below the maximum level (ML) (20 g/kg) permitted in vegetable oils (Commission Regulation (EU) No 2023/915). These five batches of the NF were also 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 concentration of glycidyl fatty acid esters (expressed as glycidol) was below LOQ (100 µg/kg) and 3-MCPD (sum of 3-MCPD and 3-MCPD fatty acid esters, expressed as 3-MCPD) was also below LOQ (100 µg/kg) in the NF, and below the limits permitted for oils for the production of baby foods established by Commission Regulation (EU) 2023/915.

The applicant analysed five batches of the NF for marine biotoxins [domoic acid (DA), yessotoxins (YTX), azaspiracids (AZA), dinophysistoxins (DTX), okadaic acid (OA), pectenotoxins (PTX), saxitoxins (STX), gonyautoxins (GTX) and cyanotoxins (i.e. microcystins and nodularine)]. Marine biotoxins and cyanotoxins were below LOQs (LOQ for DA: < 1 mg/kg; LOQ for

YTX, AZA, DTX, OA: < 5 µg/kg; LOQ for PTX, STX and GTX: < 20 µg/kg; LOQ for cyanotoxins: < 25 µg/kg). These data indicate that the microalgal strain ATCC-20889 is not expected to produce these marine biotoxins in quantifiable amounts.

Noting the high p-anisidine values in all the analysed batches of the NF in Table 1, EFSA requested the applicant to analyse five additional batches for p-anisidine. The results, presented in Table 3, indicate that p-anisidine values were lower compared with previous batches analyses and close to 10.

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

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

TABLE 1 Batch-to-batch analysis of the NF (after the addition of sunflower oil and after deodorisation).

Parameter	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Method of analysis
Proximate analysis						
Fat (g/100 g)	99.6	99.7	99.6	99.8	99.5	AOAC Ch-34 – gravimetric method
Proteins (g/100 g)	< 0.625[a]	< 0.625[a]	< 0.625[a]	< 0.625[a]	< 0.625[a]	Kjeldahl method
Carbohydrates (g/100 g)	ND	ND	ND	ND	ND	AOAC 986.25 Ch-50.1.16 (by calculation)
Energy (kcal/100 g)	896	897	896	898	895	Pearson's chemical analysis
Physico-chemical parameters						
Acid value (mg KOH/g)	0.13	0.13	0.14	0.1	0.14	AOCS – Cd 3d-63
Peroxide value (m_{eq}/kg)	0.54	0.52	0.81	0.49	0.62	AOCS – Cd 8–53
Moisture and volatiles %	0.02	0.02	0.03	0.02	0.02	AOCS – Ca 2d-25
Unsaponifiable matter %	1.57	1.61	1.68	1.63	1.65	AOCS – Ca 6d-53
Free fatty acids (g/100g)	0.07	0.07	0.07	0.05	0.07	AOCS – Cd 3d-63
p-Anisidine value	19.66	11.23	14.19	14.67	12.11	Ph. Eur. Method 2.5.36
Organic volatile impurities (mg/kg)	< 5.5	< 5.5	< 5.5	< 5.5	< 5.5	In-house method – GC
Fatty acids (% FA)						
Myristic acid – C14:0	1.87	1.83	1.77	1.53	1.95	AOAC 2012.13 – GC-FID
Pentadecanoic acid – C15:0	0.57	0.73	0.55	0.60	0.71	
Palmitic acid- C16:0	27.43	29.17	30.96	29.55	32.38	
Heptadecanoic acid – C17:0	0.27	0.34	0.32	0.20	0.34	
Stearic acid – C18:0	1.48	1.62	1.86	1.56	1.52	
Oleic acid – C18:1(n-9)	9.24	8.34	4.80	8.56	4.58	
Linoleic acid – C18:2(n-6)	1.43	1.30	0.91	1.17	0.77	
Arachidic acid – C20:4	0.36	0.43	0.49	0.37	0.23	
Eicosadienoic acid – C20:2	0.26	0.23	0.23	0.23	0.22	
Eicosatrienoic acid – C20:3	0.71	0.75	0.72	0.19	0.66	
Arachidonic acid – C20:4(n-6)	0.36	0.75	0.29	0.64	0.66	
Eicosapentaenoic acid (EPA) – C20:5(n-3)	1.26	1.01	1.08	1.14	1.11	
Erucic acid – C22:1	0.44	0.49	0.52	0.20	0.20	
Docosapentaenoic acid (DPA) – C22:5(n-6)	8.71	10.05	10.97	9.86	9.78	
Docosahexaenoic acid (DHA) – C22:6(n-3)	43.2	40.7	42.1	41.7	43.0	
Lignoceric acid – C24:0	0.67	0.68	0.75	0.68	0.67	
Nervonic acid – C24:1	0.45	0.46	0.24	0.34	0.17	
Trans fatty acids (mg/kg)	< 36.3[a]	< 36.3[a]	< 36.3[a]	< 36.3[a]	< 36.3[a]	AOAC 2012.13
Sterols (mg/100 g)						
Total sterols	402.56	307.66	442.42	497.45	461.94	AOAC 994.10 –GC-FID
Cholesterol	285.57	181.13	296.63	349.69	326.13	
Sitosterol	36.68	35.45	63.95	82.14	64.03	
Stigmasterol	48.93	35.98	52.09	51.09	47.25	
Campesterol	2.44	1.85	9.53	5.43	4.78	
Brassicasterol	28.94	53.25	20.22	9.10	19.75	

TABLE 1 (Continued)

Parameter	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Method of analysis
Heavy metals (mg/kg)						
Mercury	< 0.01[a]	< 0.01[a]	< 0.01[a]	< 0.01[a]	< 0.01[a]	AOAC, 18th edition Chp-9 – ICP
Cadmium	< 0.01[a]	< 0.01[a]	< 0.01[a]	< 0.01[a]	< 0.01[a]	
Arsenic	< 0.01[a]	< 0.01[a]	< 0.01[a]	< 0.01[a]	< 0.01[a]	
Lead	< 0.01[a]	< 0.01[a]	< 0.01[a]	< 0.01[a]	< 0.01[a]	
Copper	< 0.01[a]	< 0.01[a]	< 0.01[a]	< 0.01[a]	< 0.01[a]	
Microbiological analysis						
Total aerobic count (CFU/g)	< 10	< 10	< 10	< 10	< 10	IS 5402:2012
Yeast and moulds (CFU/g)	< 10	< 10	< 10	< 10	< 10	IS 5403:1999
Coliform count (CFU/g)	< 10	< 10	< 10	< 10	< 10	IS 5401 (P1):2012
E. coli (per 1 g)	Absent	Absent	Absent	Absent	Absent	IS 5887 (PA):1976
Salmonella (per 25 g)	Absent	Absent	Absent	Absent	Absent	IS 5587 (P3):1999
Enterobacteriaceae (CFU/mL)	< 1	< 1	< 1	< 1	< 1	ISO 21528:2017
Cronobacter sakazakii (per 10 mL)	Absent	Absent	Absent	Absent	Absent	ISO 22964:2017
Staphylococcus aureus (CFU/mL)	< 1	< 1	< 1	< 1	< 1	ISO 6888-1:1999
Process contaminants						
Glycidyl fatty esters, expressed as glycidol (µg/kg)	< 100[a]	< 100[a]	< 100[a]	< 100[a]	< 100[a]	ISO 18363-4 (2021-01) – GC-MS/MS
Sum of 3-MCPD and 3-MCPD fatty esters, expressed as 3-MCPD (µg/kg)	< 100[a]	< 100[a]	< 100[a]	< 100[a]	< 100[a]	In-house method – GC-MS

Abbreviations: AOAC, Association of Official Analytical Collaborations; AOCS, American Oil Chemists Society; CFU, Colony Forming Unit; DHA, Docosahexaenoic acid; DPA, Docosapentaenoic acid; EPA, Eicosapentaenoic acid; FA fatty acids; GC, gas chromatography; GC-FID, gas chromatography with flame ionisation detector; GC–MS, Gas Chromatography with Mass Spectroscopy; GC-MS/MS, Gas Chromatography Tandem Mass Spectrometry; ICP, Inductively Coupled Plasma; IS: Indian Standards; ISO, International Organization for Standardization; MCPD, Monochloro-Propanol-1,2-Diol; ND, not determined.

[a]LOQ, limit of quantification.

TABLE 2 In process analyses of the NF (prior to the addition of sunflower oil and prior to deodorisation).

	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5
Acid value (mg KOH/g)	0.12	0.11	0.12	0.18	0.12
Peroxide value (m_{eq}/kg)	0.58	0.54	0.86	0.55	0.68
p-Anisidine value	10.48	10.51	11.86	9.83	10.98
pH	6.6	6.6	6.5	6.5	6.6
DHA content (%)	45.32	43.06	42.75	44.22	43.61

Abbreviations: DHA, docosahexaenoic acid.

TABLE 3 P-Anisidine analyses (after addition of sunflower oil).

Batch No	p- Anisidine value (before deodorisation)	p- Anisidine value (after deodorisation)
Batch 6	12.60*	12.50*
Batch 7	10.75*	9.07
Batch 8	12.56*	12.27*
Batch 9	9.48	9.40
Batch 10	9.92	9.85

*Value above the proposed specification of 10.

3.4.1 | Stability

3.4.1.1 | *Stability of the NF*

The applicant provided stability studies with three batches of the NF (after addition of sunflower oil and after deodorisation) stored at –30°C and at 4°C up to 18 months and at 25°C and at 60% relative humidity (RH) up to 6 months. Acid values, peroxide values and DHA contents were within the limits defined in the specifications.

In order to address potential secondary oxidation, considering the relatively high p-anisidine values ranging from 11.2 to 19.7 observed in the batch-to batch analysis (Table 1), the applicant provided a report on the stability of one batch of the NF, which showed that p-anisidine value reached a value of 11.1 after 24 months of storage at 4–6°C and of 13.1 after 36 months of storage at –20°C. Upon EFSA's request, the applicant provided an analysis of three additional batches of the NF, which showed p-anisidine values around 10.2 (after 24 months) and around 10.7 (after 36 months) at storage conditions of –20°C. In all batches, peroxide, acid values and DHA content remained within the limits defined in the specifications.

Based on the stability studies provided, the applicant proposed a shelf-life for the NF of 24 months from the date of manufacture, to be stored at –20°C, under nitrogen atmosphere. The Panel considers that the stability data provide sufficient information with respect to the stability of the NF up to 24 months under the proposed storage conditions.

3.4.1.2 | *Stability of the NF ingredient under the intended conditions of use (i.e. when the NF is powdered to be incorporated into IF and FOF)*

According to the conditions of use proposed by the applicant, the NF is intended to be incorporated into IF and FOF. The NF is microencapsulated into a powder form before being incorporated into IF and FOF. The applicant provided information on the stability of the NF when undergoing the process of powdering and the storage of the powder.

The applicant provided a stability report on two batches of the NF, which underwent microencapsulation. The batches were tested for p-anisidine value, peroxide value, free FAs and DHA content. When stored at 25°C (60% RH) up to 6 months, p-anisidine values reached 9.9. When stored at 5°C up to 36 months, p-anisidine values reached 10.5. Peroxide and DHA content complied with specifications. Free FAs reached up to 0.40%. Following EFSA's request, the applicant performed additional stability tests on four batches of the microencapsulated NF stored at 5°C for 36 months. The p-anisidine values increased up to 9.6 at the end of the storage period. DHA content, peroxide and acid values were within specifications.

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

3.5 | Specifications

The specifications of the NF are presented in Table 4 and are in line with the specifications currently authorised in the Union list for *Schizochytrium* sp. oil. Considering that secondary oxidation products (such as α,β- unsaturated carbonyl compounds, malondialdehyde) may be of safety concern (Vieira et al., 2017; Kanner, 2007), the Panel proposes to add p-anisidine value in the specifications of the NF. Considering the European Pharmacopoeia value defined for salmon oils (2023) and the compositional data, the Panel recommends a maximum limit of 10 should be used for the p-anisidine value in *Schizochytrium* oils.

TABLE 4 Specifications of the NF.

Parameter (unit)	Limit
Acid value (mg KOH/g)	≤ 0.5
Peroxide value (m_{eq}/kg oil)	≤ 5.0
Moisture and volatiles (%)	≤ 0.05
Unsaponifiables (%)	≤ 4.5
Trans-fatty acids (%)	≤ 1.0
DHA-content (%)	≥ 32
p-Anisidine value	≤ 10

Abbreviations: DHA, Docosahexaenoic acid.

The applicant proposed to amend the current specifications of the Union list by increasing the DHA content from 32% to 35%. The Panel considers that the proposed changes in the specifications are not necessary from a safety point of view.

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

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

3.6.1 | History of use of the source

The source of the NF is a microalga belonging to the genus *Schizochytrium*. Table 5 presents the different entries referring to oils from microalgae of the genus *Schizochytrium* which are authorised in the Union list.

TABLE 5 Overview of the entries referring to oils from the genus *Schizochytrium* which are authorised in the Union list.

Novel food	Year of 1st authorisation	Decisions	Remarks
Schizochytrium sp. oil	2003	Decision 2003/427/EC[5]	Authorised to be added to foods but not in IF and FOF
Schizochytrium sp. oil rich in DHA and EPA	2012	Assessed by UK and authorised under Regulation (EC) No. 258/97[6]	Authorised to be added to foods but not in IF and FOF
Schizochytrium sp. (ATCC PTA-9695) oil	2015	Decision (EU) 2015/545[7]	Authorised use in IF and FOF
Schizochytrium sp. (T18) oil	2017	Assessed by UK and authorised under Regulation (EC) No. 258/97	Authorised use in IF and FOF
Schizochytrium limacinum (WZU477) oil	2021	Regulation (EU) 2021/670[8]	Authorised use in IF and FOF
Schizochytrium limacinum (FCC-3204) oil	2021	Regulation (EU) 2021/1326[9]	Authorised use in IF and FOF and in food supplements
Schizochytrium limacinum (strain TKD-1)	2024	Regulation (EU) 2024/2049[10]	Authorised use in IF and FOF
Schizochytrium sp. (strain CABIO-A-2)	2024	Regulation (EU) 2024/2101[11]	Authorised use in IF and FOF

Abbreviations: ATCC, American Type Culture Collection; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FOF, follow-on formula; IF, infant formula; UK, United Kingdom.

3.6.2 | History of use of the NF

The NF application under assessment is an extension of use for the oil referred to as *Schizochytrium* sp. oil, which is authorised to be used as food supplements and food ingredient in a wide range of products (e.g. milk-based drinks and similar products intended for young children; processed cereals-based food and baby foods for infants and young children; bakery products; cereal bars; breakfast cereals; fruit and vegetables puree).

The two strain-specific *Schizochytrium* sp. (ATCC PTA-9695) oil and *Schizochytrium* sp. (T18) oil were authorised for the same food categories as the generic *Schizochytrium* sp. oil plus for the use in IF and FOF. Oils from other *Schizochytrium* strains have been authorised to be used in IF and FOF in accordance with Regulation (EU) 609/2013 (see Table 5).

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 proposed 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 presence of DHA to IF and FOF at levels ranging between 4.8 and 12 mg/100 kJ (equation 20–50 mg/100 kcal). Considering a standard energy content of maximum 70 kcal per 100 mL of IF/FOF defined in Regulation (EU) 2016/127, the DHA concentration in the reconstituted formula is expected to range between 14 and 35 mg DHA/100 mL. Considering a minimum DHA concentration of 350 mg DHA/g in the NF, the use level for the NF corresponds to 40–100 mg NF/100 mL of the reconstituted IF or FOF, to reach the target of 14–35 mg DHA/100 mL.

[5]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.

[6]Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients.

[7]Commission Implementing Decision (EU) 2015/545 of 31 March 2015 authorising the placing on the market of oil from the microalgae *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.

[8]Commission Implementing Regulation (EU) 2021/670 of 23 April 2021 authorising the placing on the market of *Schizochytrium* sp. (WZU477) 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 141, 26.04.2021, p. 14–18.

[9]Commission Implementing Regulation (EU) 2021/1326 of 10 August 2021 authorising the placing on the market of *Schizochytrium* sp. (FCC-3204) 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 288, 11.08.2021, p. 24–27.

[10]Commission Implementing Regulation (EU) 2024/2049 of 30 July 2024 authorising the placing on the market of *Schizochytrium limacinum* (TKD-1) oil as a novel food and amending Implementing Regulation (EU) 2017/2470. *OJ L, 2024/2049, 31.7.2024*.

[11]Commission Implementing Regulation (EU) 2024/2101 of 30 July 2024 authorising the placing on the market of *Schizochytrium* sp. (CABIO-A-2) oil as a novel food and amending Implementing Regulation (EU) 2017/2470. OJ *L, 2024/2101, 31.7.2024*.

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, the intake of DHA for infants and young children fed with IF and FOF added with the NF at the proposed use levels is within the range foreseen by the Regulation.

Other DHA oils from the microalgae belonging to the genus *Schizochytrium* are currently authorised for use in IF and FOF, with use levels also in line with Regulation (EU) 2016/127 (Table 5). The NF under assessment is proposed by the applicant as an alternative source of DHA in IF and FOF. Consequently, the intended uses in IF and FOF under assessment are not expected to modify the current daily intake of DHA oil for infants and young children.

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-fatty acids were below the LOQ 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 (Section 3.4). DHA is an essential nutrient for infants and children. When used in accordance with the proposed use levels, the NF can enrich the composition of IF and FOF as set by Regulation (EU) 2016/127.

The concentration of sterols in the NF ranges between 3076 and 4974 mg/kg and corresponds to 0.0031–0.0050 mg/mL in IF and FOF to which the NF has been added (100 mg NF/100 mL). The concentration of sterols in IF and FOF containing 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 FA profile of the NF shows the presence of other components that might affect the overall ratio of FAs in IF and FOF. However, it falls under the responsibility of the manufacturers to guarantee that the overall ratio of FAs 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.

3.10 | Toxicological information

3.10.1 | Qualified presumption of safety (QPS)

The available evidence indicates that the source organism (strain ATCC-20889) belongs to the species *Schizochytrium limacinum* (basonym of *Aurantiochytrium limacinum)*. In 2020, *Schizochytrium 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. 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

Marine biotoxins in the NF were reported to be below their LOQs (Section 3.4). The Panel notes that the theoretical intakes resulting from the occurrence of marine biotoxins at their respective LOQs remained below the acute reference dose of the corresponding biotoxins (EFSA CONTAM Panel, 2009).

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

No toxicity studies that were conducted with the NF under assessment (oil produced from strain *Schizochytrium limacinum* ATCC-20889) have been provided by the applicant.

However, the toxicity of DHA 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 other DHA algal oils from *Schizochytrium* sp. 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 IF and FOF. 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, 2012a, 2012b) have also been considered by the UK competent authority in support of the authorisation of the NF *Schizochytrium* sp. (T18) in IF and FOF 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 an 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 retrieved from the literature indicated the same outcome for a diversity of DHA oils produced from other strains of *Schizochytrium* sp. (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 under assessment, taking into account the results on toxicity in studies performed with various forms of DHA oils derived from strains belonging to the genus *Schizochytrium* sp., the QPS status of the source of the NF (*Schizochytrium limacinum*), the data on the production process and on the composition of the NF and the absence of viable cells, the Panel considers that there are no concerns with regard to toxicity of the NF.

3.11 | Allergenicity

The NF is mainly composed of lipids (99.5%–99.8%). The applicant provided a proximate analysis of the NF, which indicates that proteins were below the LOQ (0.625 g/100 g) in the batch-to-batch analysis (Section 3.4 Compositional data).

The Panel considers that the NF is unlikely to trigger allergic reactions in the target population under the proposed conditions of use.

4 | DISCUSSION

The NF, which is the subject of this application, is an oil derived from the microalga *Schizochytrium* sp. (strain ATCC-20889). The available evidence indicates that the strain of the source organism ATCC-20889 belongs to the species *Schizochytrium limacinum*. The source organism which is assessed in this application is *Schizochytrium limacinum* (strain ATCC-20889) and not the generic *Schizochytrium* sp.

In 2020, *Schizochytrium limacinum* was assessed by the EFSA BIOHAZ Panel and attributed the QPS status with the qualification 'for production purposes only', which implies the absence of viable *Schizochytrium* cells in final products. The data provided by the applicant demonstrated the absence of viable cells in the NF. The Panel considers that the information provided on the production process and composition is sufficient and does not raise safety concerns.

The NF is a mixture of triglycerides in which DHA represents 40%–43% of the FAs. The applicant intends to market the NF as an ingredient in IF and FOF. The use levels proposed by the applicant were derived from Regulation (EU) 2016/127, which states the mandatory presence of DHA in IF and FOF at the level of 20–50 mg/100 kcal.

No toxicological studies were performed with the NF. However, based on the available toxicological studies on oils derived from *Schizochytrium* sp., the QPS status of the source of the NF (*Schizochytrium limacinum*), the production process, the composition of the NF, the absence of marine biotoxins and viable cells in the NF, the Panel considers that there are no concerns with regard to the toxicity of the NF.

5 | CONCLUSIONS

The Panel concludes that the NF, i.e. oil produced from the strain ATCC-20889 belonging to species *Schizochytrium limacinum*, is safe under the proposed conditions of use. The target population is infants and young children.

5.1 | Protection of proprietary data in accordance with article 26 of Regulation (EU) 2015/2283

The Panel could not have reached the conclusion on the safety of the NF under the proposed conditions of use without the data claimed as proprietary by the applicant as listed in Appendix A.

6 | STEPS TAKEN BY EFSA

1. On 10/10/23, EFSA received a letter from the European Commission with the request for a scientific opinion on the safety of the change of conditions of use of *Schizochytrium* sp. (ATCC-20889) oil as a novel food Ref. Ares(2023)6878770–10/10/2023.
2. On 10/10/23, a valid application on *Schizochytrium* sp. (ATCC-20889), which was submitted by BioPlus Life Sciences, was made available to EFSA by the European Commission through the Commission e-submission portal (NF-2023-16,402) and the scientific evaluation procedure was initiated.
3. On 20/02/2024, 04/07/2024 and 20/09/24, EFSA requested the applicant to provide additional information to accompany the application and the scientific evaluation was suspended.
4. On 14/06/2024, 30/08/2024 and 27/09/2024, additional information was provided by the applicant through the Commission e-submission portal and the scientific evaluation was restarted.
5. During its meeting on 28/11/2024, the NDA Panel, having evaluated the data, adopted a scientific opinion on the safety of oil from *Schizochytrium limacinum* (strain ATCC-20889) for use in infant and follow-on formula as a NF pursuant to Regulation (EU) 2015/2283.

ABBREVIATIONS

ADME	absorption, distribution, metabolism and excretion
AI	Adequate Intake
ANI	average nucleotide identity
Anses	Agence française de sécurité sanitaire de l'alimentation, de l'environnement et du travail (French Agency for Food, Environmental and Occupational Health and Safety)
ATCC	American Type Culture Collection
BIOHAZ	EFSA Panel on Biological Hazards
AZA	azaspiracids
bp	base pair
bw	body weight
CONTAM	EFSA Panel on Contaminants
DHA	docosahexaenoic acid
DA	domoic acid
DPA	docosapentaenoic acid
DTX	dinophysistoxins
EPA	eicosapentaenoic acid
FA	fatty acid
FOF	follow-on formula
GMP	Good manufacturing practices
GTX	gonyautoxins
HACCP	Hazard Analysis Critical Control Points
IF	infant formula
ITS	Internal Transcribed Spacer
LOQ	limit of quantification
MCPD	Monochloro-Propanol-1,2-Diol
ML	Maximum level
NCBI	National Center for Biotechnology Information
NDA	EFSA Panel on Nutrition, Novel Foods and Food Allergens
NF	novel food
OA	okadaic acid
PTX	pectenotoxins
PUFA	polyunsaturated fatty acid
QPS	Qualified presumption of safety
RH	Relative humidity
rRNA	ribosomal ribonucleic acid
STX	saxitoxins
UK	United Kingdom

WGS whole genome sequence
YTX yessotoxins

REQUESTOR

European Commission

QUESTION NUMBER

EFSA-Q-2023-00410

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APPENDIX A

List of elements of the dossier for which data protection was requested by the applicant

NF section	Elements of the application dossier for which a request for data protection was filed by the applicant	File considered as pertinent for the NDA panel to reach the conclusion on the safety (yes/No)
Identity	Annex 3 – Purchase order ATCC 20889	Yes
	Annex 4 – *Schizochytrium* whole genome sequencing report	Yes
	Annex 46 – Identification of organism using 18S rRNA gene sequence	Yes
	Annex 47 – Purchase order and Product information sheet S. limacinum MYA-1381	Yes
	Annex 64 – EGI Final Report GA221937 ANI Analysis comparison public sequences	Yes
	Annex 67 – EGI Final Report Phylogenetic tree with reference genes	Yes
Production process	Annex 5 – Water analysis	Yes
	Annex 6 – In process routine parameters tested during the production process	Yes
	Annex 7 – In process analysis during the production process	Yes
	Annex 11 – Certificates of analysis of 5 independent batches (dioxin and nutritional analysis)	Yes
	Annex 12 – Lack of microorganisms	Yes
	Annex 12bis – Method for microorganism analysis	Yes
	Annex 17 – HACCP plan	Yes
	Annex 18 – Hazard identification	Yes
	Annex 48 – Food contact material production process declaration	Yes
	Annex 49 – Media composition and CoAs	Yes
	Annex 50 – CoA Ethyl acetate	Yes
	Annex 52 – Process Flow Chart updated	Yes
	Annex 53 – Absence of *Schizochytrium*	Yes
	Annex 70 – Protocol Absence viable *Schizochytrium*	Yes
	Annex 71 – Report Evaluation of absence of *Schizochytrium*	Yes
Compositional data	Annex 22 – Certificates of analysis of 5 independent batches (chemical, elemental analysis, fatty acids profile and microbiological analysis)	Yes
	Annex 23 – Contaminants	Yes
	Annex 24 – Trans fatty acids limit of quantification	Yes
	Annex 25 – Certificates of analysis of 5 independent batches for fatty acid content	Yes
	Annex 26 – Certificates of analysis of 5 independent batches for sterol and microbiology analysis	Yes
	Annex 27 – Method of analysis of organic volatile impurities	Yes
	Annex 28 – Confirmation LOQ for organic volatile impurities	Yes
	Annex 29 – Specification sheet	Yes
	Annex 30 – Certificates of analysis of 5 independent batches (heavy metals)	Yes
	Annex 31 – Certificates of analysis of 5 independent batches for glycidol content	Yes
	Annex 32 – Certificates of analysis of 5 independent batches (3-MCPD)	Yes
	Annex 33 – Method of analysis of MCPD	Yes
	Annex 36 – Certificates of analysis of 5 independent batches for marine toxin	Yes
	Annex 37 – Stability study	Yes
	Annex 38 – Stability study for p-anisidine	Yes
	Annex 39 – DHA infant formula specifications	Yes
	Annex 40 – Composition of the 10% DHA microencapsulated powder	Yes
	Annex 41 – Composition of the 17% DHA microencapsulated powder	Yes
	Annex 42 – Stability test for the 10% DHA microencapsulated powder	Yes
	Annex 43 – Stability test for the 17% DHA microencapsulated powder	Yes
	Annex 54 – PAH analysis April 2024	Yes
	Annex 55 – Method p-anisidine measurement	Yes

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NF section	Elements of the application dossier for which a request for data protection was filed by the applicant	File considered as pertinent for the NDA panel to reach the conclusion on the safety (yes/No)
	Annex 56 – P-anisidine method verification protocol result	Yes
	Annex 59 – DHA oil 3 batches Stability –20°C 36M data	Yes
	Annex 60 – 10% DHA Powder Stability data	Yes
	Annex 61 – 17% DHA Powder Stability data	Yes
	Annex 62 – Stability Microbiology DHA Powder 10%	Yes
	Annex 63 – Stability Microbiology DHA Powder 17%	Yes