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SCIENTIFIC OPINION

Safety of oil from *Schizochytrium* sp. (strain CABIO-A-2) 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. (strain CABIO-A-2) oil as a novel food (NF) pursuant to Regulation (EU) 2015/2283. *S.* sp. is a single-cell microalga. The NF is a mixture of triglycerides in which docosahexaenoic acid (DHA) represents 38%–44% of fatty acids. The applicant proposed to use the NF 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 addition of DHA to IF and FOF at the level of 20–50 mg/100 kcal. The evidence provided demonstrated that the strain *S.* sp. CABIO-A-2 is phylogenetically closely related to the strain *S.* sp. ATCC 20888. The assessment of some already authorised *S.* sp. oils in the Union list were also based on similarities with the strain ATCC 20888. The applicant provided a 90-day repeated dose toxicity study in rats with the NF. No adverse effects were observed up to the highest dose tested, i.e. 10.2 g/kg body weight (bw) per day. Taking into account the toxicity studies performed with the NF and with DHA-oils derived from strains belonging to the genus *Schizochytrium*, its phylogenetical profile, the production process, the composition of the NF and 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

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

CONTENTS

1 | INTRODUCTION

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

On 19 March 2021, the company CABIO Biotech (Wuhan) Co., Ltd. submitted a request to the European Commission in accordance with Article 10 of Regulation (EU) 2015/2283[1] to authorise the placing on the Union market of *Schizochytrium* sp. (CABIO-A-2) oil as a novel food.

The application requests to authorise use of *Schizochytrium* sp. (CABIO-A-2) oil in infant formula (IF) and follow-on formula (FOF) as defined in Regulation (EU) No 609/2013.

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. (CABIO-A-2) oil as a novel food.

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 (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 fatty acids (FA)) 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.

Administrative and scientific requirements for NF applications referred to in Article 10 of Regulation (EU) 2015/2283 are listed in the Commission Implementing Regulation (EU) 2017/2469.[2]

A common and structured format on the presentation of NF applications is described in the EFSA Guidance on the preparation and presentation of a NF application (EFSA NDA Panel, 2016). As indicated in this guidance, it is the duty of the applicant to provide all of the available (proprietary, confidential and published) scientific data, (including both data in favour and not in favour) that are pertinent to the safety of the NF.

This NF application 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 (EFSA NDA Panel, 2016) and the principles described in the relevant existing guidance documents from the EFSA Scientific Committee. The legal provisions for the assessment are laid down in Article 11 of Regulation (EU) 2015/2283 and in Article 7 of Commission Implementing Regulation (EU) 2017/2469.

[1]Regulation (EU) 2015/2283 of the European Parliament and of the Council of 25 November 2015 on novel foods, amending Regulation (EU) No 1169/2011 of the European Parliament and of the Council and repealing Regulation (EC) No 258/97 of the European Parliament and of the Council and Commission Regulation (EC) No 1852/2001. OJ L 327, 11.12.2015, p. 1.

[2]Commission Implementing Regulation (EU) 2017/2469 of 20 December 2017 laying down administrative and scientific requirements for applications referred to in Article 10 of Regulation (EU) 2015/2283 of the European Parliament and of the Council on novel foods. OJ L 351, 30.12.2017, pp. 64–71.

The 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 the application, is an oil which is produced by the microalgae *Schizochytrium* sp. (strain CABIO-A-2). 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 microalgae followed by extraction and refinement of the oil produced by the microalgae. The main component of the oil is a mixture of triglycerides in which DHA represents 38%–44% of fatty acids. The NF 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 microalgae *S.* sp. (strain CABIO-A-2). The main component of the oil is a mixture of triglycerides mainly composed of PUFA, in which DHA is the predominant one (38%–44%), making up together with docosapentaenoic (DPA; n-6), palmitic acid and oleic acid around 72%–80% of total fatty acids in the NF.

The applicant indicated that the oil is derived from a non-genetically modified strain of *S.* sp. (strain CABIO-A-2) which was isolated from waters of the East China Sea. The strain has been deposited in the China Centre for Type Culture Collection with the deposition number M 2020393.

The applicant conducted a BLAST search with the *actin* gene sequence and the 18S rRNA gene sequence of the strain *S.* sp. CABIO-A-2 in the NCBI database (National Center for Biotechnology Information). The highest percentages of identity (> 99%) were with *actin* genes and 18S rRNA genes of the strain *S.* sp. ATCC 20888. The Panel notes that the percentage of identity against strains of known species (like *S. limacinum*) was around 90%. The applicant also provided a phylogenetic tree mapping which indicated that the strain *S.* sp. CABIO-A-2 was close to *S.* sp. ATCC 20888.

The Panel notes that the first assessment of an oil from *S.* sp., which is currently authorised in the Union List to be added to several foods but not in IF and FOF (see Section 3.6), referred to a modified strain from the wild-type parent strain ATCC 20888, which was isolated from estuarine water in California.

Although the evidence provided by the applicant does not show to which species the strain *S.* sp. (strain CABIO-A-2) belongs, the Panel notes that the 18S rRNA gene of the strain *S.* sp. (strain CABIO-A-2) is almost identical (99.88% percentage of identity) to the 18S rRNA gene of the strain *S.* sp. ATCC 20888 meaning that these two strains are phylogenetically closely related (EFSA FEEDAP Panel, 2018).

3.3 | Production process

The production process starts with inoculating culture medium with a glycerol stock of *S.* sp. CABIO-A-2 and expanding the culture until enough biomass is generated to allow for crude oil extraction. A food-grade alkaline protease is added to extract the crude oil from the biomass by cleaving proteins.

The alkaline protease is derived from *Bacillus licheniformis*, which has not been genetically modified. Characteristics of the alkaline protease have been provided by the applicant (e.g. activity, pH, temperature and pH dependency, enzyme inactivation). The alkaline protease was not detected in three batches of the NF (LOD: 200 units/mL). Based on the production process and the data provided, the Panel considers that there is no indication that active enzyme will be present in the NF. The manufacturer of the alkaline protease certifies that the microorganism *B. licheniformis* is not present in the enzyme preparation. According to the cytotoxicity test provided by the applicant, the production strain *B. licheniformis* does not produce either diarrhoeagenic/enterotoxigenic or emetic toxins. Upon EFSA's request for information, the applicant provided data which demonstrated the absence of acquired antimicrobial resistance (AMR) genes which may be carried over from the microorganism used to produce the enzyme preparation to the NF. During the evaluation, the applicant informed EFSA that the strain used to produce the enzyme preparation has been re-classified from *B. licheniformis* to *Bacillus paralicheniformis*. *Bacillus paralicheniformis* was assessed and given a QPS status with the qualification of 'absence of bacitracin production ability' (EFSA BIOHAZ Panel, 2023). Upon EFSA's request of information, the applicant tested five batches of the NF for bacitracins; the sum of bacitracins A, B and C was below the detection limit (20 µg/kg).

After the alkaline protease reaction is completed, the alkaline protease is heat-inactivated and the solids are removed by centrifugation, yielding the DHA crude oil. If the crude oil does not meet quality control parameters, the batch will be subjected to additional refining steps to ensure the batch complies with quality specifications. The crude oil is then stored

in nitrogen flushed HDPE (high density polyethylene) containers at –18°C to –13°C for not more than 24 months before proceeding to the second refining steps.

The crude oil enters the step of oil refining (degumming, decolorising, deodorisation). The oil undergoes a first distillation step (molecular distillation) and then the oil is blended with antioxidants (ascorbyl palmitate, vitamin E, rosemary extracts, soy lecithin) and distilled a second time. Sunflower oil is added to standardise the DHA content. The finished oil is packaged under vacuum in heat-sealed food-grade aluminium foil bags or HDPE drums flushed with nitrogen gas to minimise oxidation and stored at temperature from –18°C to –13°C. The applicant confirmed that the production process is carried out in inert atmosphere (nitrogen).

Considering the information provided by the applicant on the absence of viable cells in the NF, the high temperature applied at certain steps of 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.

According to the information provided, the NF is produced in Food Safety System certified facilities.

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. 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 a mixture of triglycerides mainly composed of PUFA in which DHA is the predominant one (38%–44%), making up together with DPA (n-6), palmitic acid and oleic acid around 72%–80% of the fatty acids in the NF.

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 five batches of the NF (Table 1). The batch analyses presented in Table 1 were performed after the addition of sunflower oil and antioxidants. Upon EFSA's request for information, the applicant provided the analysis of additional five batches of the pure algal oil (before the addition of sunflower oil and antioxidants) which showed that the pure algal oil complies with the proposed specifications of the NF.

During the evaluation, EFSA requested clarification to the applicant on the analysis of total fat as triglycerides which ranged between 86% and 93% (Table 1). The applicant clarified that the characterisation of fat profile depends on the method applied. The applicant analysed a batch of the NF with a different method than the one reported in Table 1, which showed that the total fat as triglycerides in the NF amounted to 95.4%, 2.4% as diglycerides and 2.1% as monoglycerides. The Panel considers the clarification provided by the applicant satisfactory.

The results of analyses in batches of the NF showed that common marine biotoxins were below the respective limits of quantifications (LOQs): five batches were analysed for paralytic shellfish poisoning toxins (PSP) and yessotoxins (LOQ = 20 μg/kg); diarrhetic shellfish poisoning toxins (DSP), LOQ = 5 μg/kg); pectenotoxins and azaspiracids (LOQ = 5 μg/kg); three batches were analysed for domoic acid (LOQ = 3 μg/kg).

With regard to chemical contaminants, the concentrations of heavy metals, dioxins, polychlorinated biphenyls and polycyclic aromatic hydrocarbons reported in the 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. Three 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 FA esters) (Table 1). The maximum concentrations for these contaminants are within the limits established by Commission Regulation (EU) 2023/915.

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).

Parameter (unit)	Batch number					Method of analysis
Proximate analysis	**#1**	**#2**	**#3**	**#4**[b]	**#5**	
Total fat (%)	100	100	100	100	100	AOAC 963.15 Soxhlet extraction - gravimetry method
Proteins (%)	< 0.1[a]	< 0.1[a]	< 0.1[a]	< 0.1[a]	< 0.1[a]	AOAC 984.13
Carbohydrates (%)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	Calculated
Physico-chemical parameters	**#6**	**#2**	**#3**	**#4**[b]	**#5**	
Acid value (mg KOH/g)	0.23	0.18	0.16	0.09	0.28	ISO 660:2009
Peroxide value (meq/kg)	2.85	3.32	3.53	4.72	3.68	AOCS Cd 8b-90
Free fatty acids (calculated as oleic acid) (%)	0.12	0.09	0.08	0.05	0.14	ISO 660:2009
	#7[b]	**#8**	**#9**[b]	**#10**		
***p*-Anisidine value**	4	4	3	3	Not tested	ISO 6885
Residual impurities	**#1**	**#2**	**#3**	**#4**[b]	**#5**	
Moisture and volatile matter (%)	< 0.01[a]	0.01	0.01	0.01	0.01	ISO 662:2016
Ash (%)	< 0.01[a]	0.04	0.03	< 0.01[a]	< 0.01[a]	AOAC 923.03
	#6	**#2**	**#3**	**#4**[b]	**#5**	
Unsaponifiable matter (%)	0.85	0.82	0.75	0.59	0.65	ISO 18609
Solvent residue (hexane) (mg/kg)	< 1[a]	< 1[a]	< 1[a]	< 1[a]	< 1[a]	HS-GC–MS
Fatty acids (g/100 g)	**#11**	**#1**	**#3**	**#5**	**#12**	AOCS Ce 1b-89 + AOAC 996.06 GC-FID
Butyric acid – C4:0	< 0.02[a]	< 0.02[a]	0.02	< 0.02[a]	0.03	
Caproic acid – C6:0	< 0.02[a]	< 0.02[a]	< 0.02[a]	< 0.02[a]	< 0.02[a]	
Caprylic acid – C8:0	< 0.02[a]	< 0.02[a]	< 0.02[a]	< 0.02[a]	< 0.02[a]	
Capric acid – C10:0	< 0.02[a]	< 0.02[a]	< 0.02[a]	< 0.02[a]	< 0.02[a]	
C11:0 Undecanoic acid	< 0.02[a]	< 0.02[a]	< 0.02[a]	< 0.02[a]	< 0.02[a]	
Lauric acid – C12:0	0.04	0.07	0.05	0.04	0.04	
Myristic acid – C14:0	0.68	2.56	1.03	0.50	0.58	
Myristoleic acid – C14:1	< 0.02[a]	< 0.02[a]	< 0.02[a]	< 0.02[a]	< 0.02[a]	
Pentadecanoic acid – C15:0	0.08	0.26	0.16	0.07	0.10	
Pentadecenoic acid – C15:1	< 0.02[a]	< 0.02[a]	< 0.02[a]	< 0.02[a]	< 0.02[a]	
Palmitic acid – C16:0	15.97	14.71	18.71	21.80	19.82	
Palmitoleic acid – C16:1	0.10	0.35	0.15	0.14	0.12	
Margaric acid – C17:0	0.06	0.09	0.09	0.09	0.12	
Stearic acid – C18:0	1.23	0.83	1.25	1.68	1.55	
Vaccenic Acid – C18:1n-7	0.17	0.52	0.21	0.21	0.19	
Oleic acid – C18:1(n-9)	9.56	5.52	4.81	6.45	6.01	
Linoleic acid – C18:2(n-6)	0.96	1.35	0.72	0.91	0.88	
γ-Linolenic acid – C18:3(n-6) (GLA)	0.12	0.20	0.17	0.12	0.12	
α-Linolenic acid – C18:3(n-3) (ALA)	0.18	0.15	0.17	0.22	0.19	
Octadecatetraenoic acid – C18:4	0.19	0.26	0.24	0.20	0.18	
Arachidic acid – C20:0	0.28	0.17	0.25	0.31	0.31	
Gondoic acid – C20:1n-9	0.04	0.02	0.03	0.03	0.02	
C20:2(n-6)	< 0.02[a]	< 0.02[a]	< 0.02[a]	< 0.02[a]	< 0.02[a]	
C20:3(n-3)	< 0.02[a]	< 0.02[a]	< 0.02[a]	< 0.02[a]	< 0.02[a]	
C20:3(n-6)	< 0.02[a]	0.33	0.27	0.20	0.23	
Eicosatrienoic acid – C20:3(n-3)	< 0.02[a]	< 0.02[a]	< 0.02[a]	< 0.02[a]	< 0.02[a]	

TABLE 1 (Continued)

Parameter (unit)	Batch number					Method of analysis
Eicosatrienoic acid – C20:3(n-6)	0.23	0.33	0.27	0.20	0.23	
C20:4(n-3)	0.60	0.69	0.65	0.54	0.56	
Arachidonic acid – C20:4(n-6)	0.18	0.44	0.39	0.54	0.51	
Eicosapentaenoic acid – C20:5(n-3) (EPA)	0.40	0.63	0.62	0.62	0.54	
Heneicosapentaenoic acid – C21:5n-3	< 0.02[a]	< 0.02[a]	< 0.02[a]	< 0.02[a]	< 0.02[a]	
Behenic acid C22:0	0.26	0.15	0.19	0.24	0.24	
Erucic acid – C22:1(n-9)	< 0.02[a]	0.33	< 0.02[a]	< 0.02[a]	< 0.02[a]	
Docosadieonic acid – C22:2(n-6)	< 0.02[a]	< 0.02[a]	< 0.02[a]	< 0.02[a]	< 0.02[a]	
Docosapentaenoic acid – C22:5(n-3) (DPA n-3)	0.15	0.46	0.27	0.10	0.16	
Docosapentaenoic acid – C22:5(n-6) (DPA n-6)	11.48	12.64	11.68	9.59	10.54	
Docosahexaenoic acid – C22:6(n-3) (DHA)	43.85	38.80	43.88	42.93	42.71	
Lignoceric acid – C24:0	0.17	0.20	0.19	0.22	0.21	
Nervonic acid – C24:1	< 0.02[a]	0.12	< 0.02[a]	< 0.02[a]	< 0.02[a]	
Total fat as triglycerides (g/100 g)	91.61	85.97	91.00	92.38	90.15	
Total saturated fatty acids (g/100 g)	18.79	19.05	21.93	24.97	23.00	
Total trans-fatty acids (g/100 g)	0.31	0.13	0.31	0.24	0.16	
Sterols (mg/100 g)	**#11**	**#1**	**#13**	**#13**	**#12**	
Total plant sterols and stanols in fat	635	1075	752	843	615	NMKL 198:2014
Brassicasterol	40	183	80	78	20	
Cholesterol	299	449	320	403	259	
Campesterol	15	4	17	24	13	
Campestanol	2	5	2	3	2	
Stigmasterol	94	407	149	161	64	
Unidentified sterols	343	303	340	404	388	
Sitosterol	77	68	67	73	63	
Sitostanol+delta-5-avenasterol	5	43	29	42	9	
Delta-5, 24-stigmastadienol	12	9	15	16	12	
Delta-7-stigmastenol	41	41	43	36	37	
Delta-7-avenasterol	6	10	10	6	5	
Cycloartenol	6	9	7	8	4	
24-Methylenecycloartanol	2	2	1	4	3	
Citrostadienol	4	4	3	2	3	
Metals (mg/kg)	**#1**	**#2**	**#3**	**#4**[b]	**#5**	
Lead	< 0.05[a]	< 0.05[a]	< 0.05[a]	< 0.05[a]	< 0.05[a]	BS EN ISO 17294-22,016 mod.
Arsenic	< 0.05[a]	< 0.05[a]	< 0.05[a]	< 0.05[a]	< 0.05[a]	BS EN ISO 17294-22,016 mod.
Mercury	< 0.005[a]	< 0.005[a]	< 0.005[a]	< 0.005[a]	< 0.005[a]	BS EN 13806:2002
Cadmium	< 0.01[a]	< 0.01[a]	< 0.01[a]	< 0.01[a]	< 0.01[a]	BS EN ISO 17294-22,016 mod.
Copper	< 0.05[a]	< 0.05[a]	< 0.01[a]	< 0.05[a]	< 0.05[a]	DIN EN ISO 17294-2 (2017–01) mod. or AOCS Ca 17–01

(Continues)

TABLE 1 (Continued)

Parameter (unit)	Batch number					Method of analysis
Iron	< 0.1[a]	< 0.02[a]	< 0.02[a]	< 0.1[a]	< 0.1[a]	AOCS Ca 17–01 or ICP-MS
Microbiological analysis	**#6**	**#2**	**#3**	—	—	
Total Aerobic Plate Count (CFU/mL)	< 1.0[a]	< 1.0[a]	< 1.0[a]	—	—	ISO 4833-1:2013
Moulds (CFU/mL)	< 1.0[a]	< 1.0[a]	< 1.0[a]	—	—	ISO 21527
Yeast (CFU/mL)	< 1.0[a]	< 1.0[a]	< 1.0[a]	—	—	ISO 21527
Total Coliform Bacteria (MPN/mL)	< 0.03[a]	< 0.03[a]	< 0.03[a]	—	—	ISO 4831:2006
***Salmonella* (/25 g)**	Not detected	Not detected	Not detected	—	—	ISO 6579-1:2017
	#1	**#2**	**#3**	**#4**[b]	**#5**	
***Escherichia coli* (/25 mL)**	Not detected	Not detected	Not detected	Not detected	Not detected	ISO 16649-3:2015
Coagulase-positive staphylococci (/1 mL)	Not detected	Not detected	Not detected	Not detected	Not detected	ISO 6888-3:2003
***Bacillus cereus* (CFU/mL)**	< 1.0[a]	< 1.0[a]	< 1.0[a]	< 1.0[a]	< 1.0[a]	ISO 7932:2004
***Listeria monocytogenes* (/25 mL)**	Not detected	Not detected	Not detected	Not detected	Not detected	ISO 11290-1:2017
***Cronobacter* spp. (/25 mL)**	Not detected	Not detected	Not detected	Not detected	Not detected	ISO 22964:2017
Enterobacteriaceae (/25 mL)	Not detected	Not detected	Not detected	Not detected	Not detected	ISO 21528-1: 2017
Process contaminants	**#11**	**#14**[b]	**#3**	—	—	
Glycidyl-ester (determined as glycidol) (μg/kg)	< 100[a]	< 500[a]	< 100[a]	—	—	GC–MS
Sum of free 3-MCPD and 3-MCPD esters (determined as free 3-MCPD) (μg/kg)	< 100[a]	140	155	—	—	GC–MS

Abbreviations: AOAC, Association of Official Analytical Collaborations; AOCS, American Oil Chemists Society; CFU, colony forming unit; GC–FID, gas chromatography–flame ionisation Detection; GC–MS, gas chromatography with mass spectroscopy; HS-GC–MS, headspace-gas chromatography/mass spectrometry; ICP-MS, inductively coupled plasma-mass spectrometry; ISO, International Organization for Standardisation; MCPD, monochloro-propan-1,2-diol; MPN, most probable number; ND, not detected.

[a]LOQ, limit of quantification.

[b]These batches did not undergo winterisation.

3.4.1 | Stability

3.4.1.1 | *Stability of the NF*

The applicant performed stability tests with four batches of the NF (added with sunflower oil). The tests were carried out at –20°C for up to 24 months. The batches were analysed for DHA, peroxide and anisidine values, which complied with the proposed specifications at each time point analysed up to the end of the testing period (24 months).

Based on the stability studies provided, the applicant proposed a shelf life for the NF of 24 months, at a temperature from –18 to –13°C. 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 under the intended conditions of use*

The NF is intended to be incorporated in IF and FOF in a powdered form. The applicant indicated that the micro-encapsulation process to transform the NF, which is an oil, into a powder is performed by manufacturers of IF and FOF. During the assessment, EFSA requested information on the stability of the NF when processed into a powder. In reply, the applicant initiated a stability study with five batches of the NF in powdered form. The oxidative parameters of the powdered form tested at time 0 (free fatty acids, p-anisidine value, peroxide value) were below the limits set in the specifications.

The Panel notes that the data on oxidative parameters 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 –20°C up to 2 years, the Panel expects the NF to be stable under the intended conditions of use.

3.5 | Specifications

The specifications of the NF are presented in Table 2. Considering that secondary oxidation products (such as α,β-unsaturated carbonyl compounds, malonaldehyde) may be of safety concern (Kanner, 2007; Vieira et al., 2017), the Panel proposes to add the *p*-anisidine value in the specifications of the NF. Considering the European Pharmacopoeia value defined for salmon oils (European Pharmacopoeia, 2023) and the compositional data, a maximum limit of 10 could be used for the *p*-anisidine value in *Schizochytrium* oils.

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

TABLE 2 Specifications of the NF.

Description: The NF is an oil produced from the strain CABIO-A-2 of the microalgae *Schizochytrium* sp.	
Parameter (unit)	Limit
DHA content (%)	≥ 35.0
Acid value (mg KOH/g)	≤ 0.5
Peroxide value (meq/kg)	≤ 5.0
Moisture and volatiles (%)	≤ 0.05
Unsaponifiables (%)	≤ 3.5
Trans-fatty acids (%)	≤ 2.0
Free fatty acids (%)	≤ 0.4
***p*-Anisidine value**	≤ 10

Abbreviation: DHA, docosahexaenoic acid.

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*. Table 3 presents the different entries referring to oils from microalgae of the genus *Schizochytrium* which are authorised in the Union list.

TABLE 3 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[a]	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[b]	Authorised to be added to foods but not in IF and FOF
Schizochytrium sp. (ATCC PTA-9695) oil	2015	Decision (EU) 2015/545[c]	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[d]	Authorised use in IF and FOF
Schizochytrium limacinum (FCC-3204) oil	2021	Regulation (EU) 2021/1326[e]	Authorised use in IF and FOF

Abbreviation: UK, United Kingdom.

[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]Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients.
[c]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.
[d]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.
[e]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, 11.8.2021, p. 24–27.

This genus has been used as a source of DHA-oils since 2003, the year of the first authorisation of DHA-oil from *S.* sp. as NF. The first assessment of DHA-oil from *S.* sp. involved the strain ATCC 20888 (United Kingdom, 2002). Following two substantial equivalence assessments (FSAI, 2014; Anses, 2018), two other strains (FCC-1324 and FCC-3204, respectively) were recognised as valid sources to produce DHA-oils equivalent to the original NF. On the Union list, the DHA-oils produced from these strains are commonly referred to as '*S.* sp. oil'.

The following strains belonging to the genus *Schizochytrium* have been authorised for the production of DHA-oils to be used in IF and FOF: *S.* sp. ATCC PTA-9695, *S.* sp. T18, *S. limacinum* WZU477 (EFSA NDA Panel, 2020) and *S. limacinum* FCC-3204 (EFSA NDA Panel, 2021).

3.6.2 | History of use of the NF

In 2020, the applicant submitted a GRAS (Generally Recognised as Safe) notification to the Food and Drug Administration (FDA) for the DHA-oil from the microalgae *S.* sp. CABIO-A-2 to be used in IF and in foods (GNR No 934[3]). In 2021, this evaluation was positively finalised by the FDA and the oil was granted a GRAS status.

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. 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 addition 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 level 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.

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 authorised for use in IF and FOF, with use levels in line with Regulation (EU) 2016/127 (Table 3 in Section 3.6.1). The NF under assessment is proposed by the applicant as an alternative source of DHA for IF and FOF. Consequently, the intended uses in IF and FOF of the NF under assessment is 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 ranged between 0.13 and 0.31 g/100 g and based on the acid value, free FAs are not expected

[3]https://www.fda.gov/media/144093/download

to be of concern. The FA profile reveals that DHA is the predominant acid. 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 (20–50 mg DHA/100 kcal).

The concentration of sterols in the NF ranges between 6150 and 10,750 mg/kg which corresponds to 0.0062–0.011 mg/mL in IF and FOF added with the NF (100 mg NF/100 mL of formula). The concentration of sterols in IF and FOF added with the NF is in the range of the concentrations 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 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, the consumption of the NF is not nutritionally disadvantageous.

3.10 | Toxicological information

This section reports two studies performed with the NF (acute and 90-day repeated dose toxicity study) and other studies performed with DHA algal oils produced from different strains of *S.* sp. No genotoxicity studies with the NF were provided by the applicant. This section also addresses the absence of marine biotoxins in the NF.

3.10.1 | Acute and sub-chronic toxicity studies with the NF

The applicant provided an acute study with the NF (Unpublished study report, dated, 2012a). Neither toxic symptoms nor deaths were recorded during the 14 days observation period after administration of a single oral dose of 20 g/kg body weight (bw) of the test material to mice. However, the Panel considers that, in general, acute toxicity studies are of limited relevance for the safety assessment of novel foods.

The applicant provided a 90-day study with the NF (oil produced by the microalgae *S.* sp. CABIO-A-2) (Unpublished study report, dated, 2012b). This study was not compliant with OECD guidelines (i.e. some endpoints were missing). However, the Panel notes that this study investigated main endpoints related to body weight, liver, kidneys, spleen and testis. Thus, the Panel considers that this study can be used to inform on the safety of the NF. The test material was mixed with vegetable oil and administered by oral gavage to groups of 10 male and 10 female Wistar rats at dose levels corresponding to 0 (vegetable oil – not specified), 2.5, 5.1 and 10.2 g/kg bw daily for 90 days.

No abnormal behaviour or deaths were reported during the study. There were no statistically significant differences between control and treated groups in body weights and feed consumption. There were no statistically significant differences between groups either in the haematological parameters investigated (haemoglobin, red blood cell count, white blood cell count including lymphocytes, monocytes and granulocytes) or in the clinical biochemistry parameters investigated (alanine aminotransferase, aspartate aminotransferase, blood urea nitrogen, total cholesterol, triglyceride, creatinine, glucose, albumin, albumin/globulin ratio and total protein).

No statistically significant differences were observed between control and treated groups in either absolute or relative weights of the collected organs (liver, spleen, kidneys and testis). No gross abnormalities were observed in the heart, liver, spleen, lungs, kidneys, stomach or intestine at sacrifice. No gross abnormalities were observed in the liver, spleen, kidney, stomach, intestines, ovary and testes of the control and high-dose groups.

No histopathological findings were observed in the spleen, stomach, intestines, ovaries or testes in the high-dose group. Findings in liver and kidney in the control and treated animals with low incidence and severity were not considered treatment related by the Panel.

The Panel considers that in this study no adverse effects were observed up to the highest dose tested, i.e. 10.2 g/kg bw per day.

3.10.2 | Absence of marine biotoxins

Marine biotoxins in the NF were reported to be below their LOQs (see 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.

The toxicity of DHA algal oils produced from different strains of *S.* 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, sub-chronic toxicity studies with rats, and developmental and reproductive toxicity studies with rats, were performed with various forms of DHA algal oils from *S.* sp. Most of these studies were assessed and used to conclude on the safety of other DHA algal oils from *S.* sp. in former authorisation frameworks. 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 oil derived from *S.* 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, Tran, Peach, Bauter, & Marone, 2012; Schmitt, Tran, Peach, Edwards, & Greeley, 2012) have also been considered by the UK competent authority in support of the authorisation of the oil derived from *S.* sp. (T18) in IF and FOF in 2017 (United Kingdom, 2017). In addition, two other studies (Falk et al., 2017; Lewis et al., 2016), performed with DHA-oils from unspecified strains of *S.* 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 sub-chronic 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 *S.* sp. (Abril et al., 2003; Blum et al., 2007; Hammond et al., 2001a, 2001b, 2002; Kroes et al., 2003).

3.11 | Allergenicity

The applicant provided a proximate analysis of the NF, which indicates that proteins were below the LOQ (0.1 g/100 g). The Panel considers that the NF is unlikely to trigger allergic reactions in the general population or subgroups thereof under the proposed conditions of use.

4 | DISCUSSION

The NF, which is the subject of this application, is an oil derived from the microalgae *S.* sp. CABIO-A-2. The NF is a mixture of triglycerides in which DHA represents 38–44% of FA. 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 addition of DHA to IF and FOF at the level of 20–50 mg/100 kcal.

The evidence provided demonstrates that the strain *S.* sp. CABIO-A-2 is phylogenetically closely related to the strain *S.* sp. ATCC 20888. The Panel notes that the assessment of some already authorised *S.* sp. oils in the Union list were also based on similarities with the strain ATCC 20888 (i.e. first assessment and substantial equivalence assessments as reported in Section 3.6.1).

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

The applicant provided a 90-day repeated dose toxicity study in rats with the NF. The Panel considers that in this study no adverse effects were observed up to the highest dose tested, i.e. 10.2 g/kg bw per day.

Taking into account the toxicity studies performed with the NF and with DHA-oils derived from strains belonging to the genus *Schizochytrium*, that *S.* sp. CABIO-A-2 is phylogenetically closely related to the strain *S.* sp. ATCC 20888, the production process, the composition of the NF and 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 *S.* sp. CABIO-A-2, is safe under the proposed conditions of use. The target population is infants and young children.

6 | STEPS TAKEN BY EFSA

1. On 12/07/2021 EFSA received a letter from the European Commission with the request for a scientific opinion on the safety of *Schizochytrium* sp. (CABIO-A-2) oil as a NF. Ref. Ares(2021)4514836–12/07/2021.
2. On 12/07/2021, a valid application on *Schizochytrium* sp. (CABIO-A-2) oil, which was submitted by CABIO Biotech (Wuhan) Co., Ltd., was made available to EFSA by the European Commission through the Commission e-submission portal (NF 2021/2445) and the scientific evaluation procedure was initiated.
3. On 15/10/2021, 18/03/2022, 23/09/2022 and on 17/02/2023 EFSA requested the applicant to provide additional information to accompany the application and the scientific evaluation was suspended.
4. On 02/03/2022, 05/08/2022, 10/01/2023 and on 13/09/2023 additional information was provided by the applicant through the Commission e-submission portal and the scientific evaluation was restarted.
5. During its meeting on 26/10/2023, the NDA Panel, having evaluated the data, adopted a scientific opinion on the safety of oil from *Schizochytrium* sp. (strain CABIO-A-2) for use in IF and FOF as a NF pursuant to Regulation (EU) 2015/2283.

ABBREVIATIONS

ADME	absorption, distribution, metabolism and excretion
AI	adequate intake
AMR	anti microbial resistance
ATCC	American Type Culture Collection
BIOHAZ	EFSA Panel on Biological Hazards
bw	body weight
CONTAM	EFSA Panel on Contaminants
DHA	docosahexaenoic acid
DPA	docosapentaenoic acid
DSP	Diarrhetic Shellfish Poisoning
EPA	eicosapentaenoic acid
FA	fatty acids
FAIM	Food Additive Intake Model
FDA	Food and Drug Administration
FEEDAP	EFSA Panel on Additives and Products used in Animal Feed
FOF	follow-on formula
FSAI	Food Safety Authority of Ireland
GMP	Good Manufacturing Practice
GRAS	generally recognised as safe
HDPE	high density polyethylene
IF	infant formula
LOD	limit of detection
LOQ	limit of quantification
MCPD	Monochloro-Propan-1,2-Diol
NCBI	National Center for Biotechnology Information
NDA	EFSA Panel on Nutrition, Novel Foods and Food Allergens
NOAEL	no observed adverse effect level
NF	novel food
OECD	Organisation for Economic Co-operation and Development
PSP	paralytic shellfish poisoning
PUFA	polyunsaturated fatty acids
QPS	qualified presumption of safety
rRNA	ribosomal Ribonucleic Acid

CONFLICT OF INTEREST

If you wish to access the declaration of interests of any expert contributing to an EFSA scientific assessment, please contact interestmanagement@efsa.europa.eu.

REQUESTOR

European Commission

QUESTION NUMBER

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