Adopted: 26 October 2023

DOI: 10.2903/j.efsa.2023.8414

efsa JOURNAL

SCIENTIFIC OPINION

Safety of oil from *Schizochytrium limacinum* (strain TKD-1) 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. (TKD-1) oil as a novel food (NF) pursuant to Regulation (EU) 2015/2283. *Schizochytrium* sp. is a single-cell microalga. The strain TKD-1, used by the applicant (ATK Biotech Co. Ltd.), belongs to the species *Schizochytrium limacinum*. The NF is a mixture of triglycerides in which docosahexaenoic acid (DHA) represents 53%–61% 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. *S. limacinum* was attributed the qualified presumption of safety (QPS) status with the qualification 'for production purposes only'. Data provided by the applicant demonstrated the absence of viable cells in the NF. No toxicological studies were performed with the NF. However, based on the available toxicological data on oils derived from *Schizochytrium* sp., the QPS status of the source of the NF, the production process, the composition of the NF and the absence of marine biotoxins and viable cells in the NF, the Panel considers there are no concerns with regard to toxicity of the NF. The Panel concludes that the NF is safe under the proposed conditions of use.

KEYWORDS

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 17 December 2020, the company TK Biohealth Co., LTD. (now ATK Biotech Co. Ltd.) (China),[1] Ltd. submitted a request to the European Commission in accordance with Article 10 of Regulation (EU) 2015/2283[2] to authorise the placing on the Union market of *Schizochytrium* sp. (TKD-1) oil as a novel food.

The application requests to authorise use of *Schizochytrium* sp. (TKD-1) 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. (TKD-1) 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. 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 et al., 2020, 2023).

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.[3]

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

[1]The name of the applicant was changed to ATK Biotech Co., Ltd. (China).

[2]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.

[3]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.

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.

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 which is produced by the microalgae *Schizochytrium* sp. (strain TKD-1). 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 composed of PUFA in which DHA represents 53%–61% 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 *Schizochytrium* sp. (strain TKD-1). The main component of the oil is a mixture of triglycerides mainly composed of PUFA, in which DHA is the predominant one (53%–61%), making up together with docosapentaenoic (DPA; n-6) and palmitic acid around 90% of total FA in the NF.

The applicant confirmed that the oil is derived from a non-genetically modified strain of *Schizochytrium* sp. TKD-1 that was isolated from a decayed leaf in the East China Sea. Sufficient information was provided by the applicant on the process to isolate the strain and to obtain a pure culture free of contaminants. The strain has been deposited in the China Center for Type Culture Collection with the number M 2020378.

During the assessment of this NF, EFSA requested the applicant to identify the strain 'TKD-1' at the species level. In reply the applicant provided the complete 18S rRNA gene sequence (total length of 1786 bp) and a BLAST analysis. The BLAST analysis showed a percentage of identity of 98.75% of the 18S rRNA gene sequence of the TKD-1 strain with the strain *Aurantiochytrium limacinum* ATCC MYA 1381. *Schizochytrium limacinum* is the basonym of *Aurantiochytrium limacinum* (Yokoyama & Honda, 2007).

Based on the data provided, the Panel considers that the strain TKD-1 is a member of the species *Schizochytrium limacinum*.

3.3 | Production process

The production process starts with inoculating sterile culture medium with *Schizochytrium* sp. TKD-1 and expanding the culture to generate the biomass needed to produce the oil. The cells in the biomass are then lysed using an alkaline protease.

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). The absence of alkaline protease in the NF was measured by protease activity in five lots of the NF versus positive control (*Bacillus licheniformis* alkaline protease). Protease activity in the NF was below the detection limit (1 Unit/g). 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 *Bacillus licheniformis* is not present in the enzyme preparation. According to the cytotoxicity test provided by the applicant, the production strain *Bacillus 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 *Bacillus 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 et al., 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 lysed biomass is centrifuged to remove the cellular debris. The clarified oil is then degummed, deacidified, bleached, and deodorised. Food-grade sunflower oil and soybean-derived tocopherols are added to adjust the DHA content and prevent oxidation. The finished product is then filtered, aliquoted into aluminium barrels or drums and overlayed with nitrogen, then sealed and frozen until release.

Considering the data 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 according to the China Food Safety National Standards and in line with Hazard Analysis Critical Control Points (HACCP) principles.

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 (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 of the NF 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, making up together with DPA (n-6) and palmitic acid around 90% of total 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, the applicant provided analytical information for five batches of the NF (pure algal oil, before the addition of sunflower oil) (Table 1). Upon EFSA's request for information on the fat content in the NF, the applicant analysed the fat content in five additional batches of the NF by using a different method than the one reported in Table 1. Based on this method (GB 5009.6.2016 method II), the fat content in the NF was about 99.5–99.8 g/100 g. The Panel considers this new fat content analysis to be satisfactory.

The results of analyses in five batches of the NF showed that common marine biotoxins were below the respective limits of quantification (LOQs): paralytic shellfish poisoning toxins, saxitoxins and yessotoxins: LOQ = 20 μg/kg; diarrhetic shellfish poisoning toxins, okadaic acid, domoic acid; pectenotoxins and azaspiracids: LOQ = 5 μg/kg; amnesic shellfish poisoning: LOQ = 1 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 analyses are within the EU limits established in the respective regulations and do not present concerns from a safety point of view. The 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) (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 (before the addition of sunflower oil).

	Batch number					
Parameter (unit)	**#1**	**#2**	**#3**	**#4**	**#5**	**Method of analysis**
Macronutrient content						
Total fat (g/100 g)[b]	93.3	94.5	95.2	95.7	97.6	AOAC 996.06
Proteins (g/100 g)	< 0.1[a]	< 0.1[a]	< 0.1[a]	< 0.1[a]	< 0.1[a]	AOAC 2001.11
Total carbohydrates (g/100 g)	6.7	5.5	4.8	4.3	2.4	FDA 21 CFR 101.9
Physico-chemical parameters						
Acid value (mg KOH/g)	0.18	0.19	0.19	0.16	0.16	AOCS Cd-3d-63
Peroxide value (meq/kg)	0.78	0.79	0.72	1.10	1.04	ISO 3960:2017
p-Anisidine value	3.7	8.6	6.8	10.1[c]	10.3[c]	ISO 6885:2016
Free fatty acids (g/100 g)	0.09	0.09	0.09	0.09	0.09	AOCS Ca 5a-40
Residual impurities						
Moisture and volatiles (g/100 g)	0.01	0.01	0.01	< 0.01	< 0.01	ISO 662:2016
Unsaponifiable matter (g/100 g)	1	1	1	1.8	1.9	ISO 3596:2000
Insoluble impurities (g/100 g)	< 0.01[a]	< 0.01[a]	< 0.01[a]	< 0.01[a]	< 0.01[a]	GB/T 15688-2008
Ash (g/100 g)	< 0.01[a]	< 0.01[a]	< 0.01[a]	< 0.01[a]	< 0.01[a]	AOAC 930.05

(Continues)

TABLE 1 (Continued)

Parameter (unit)	Batch number #1	#2	#3	#4	#5	Method of analysis
Fatty acids (g/100 g)						
Lauric acid – C12:0	0.06	0.06	0.06	0.06	0.06	AOAC 996.06
Myristic acid – C14:0	0.54	0.55	0.55	0.50	0.50	
Pentadecanoic acid – C15:0	0.08	0.08	0.08	0.06	0.06	
Palmitic acid – C16:0	18.36	18.09	18.33	16.22	16.28	
Heptadecanoic acid – C17:0	0.07	0.08	0.08	0.07	0.07	
Stearic acid – C18:0	1.02	1.1	1.06	0.98	0.98	
Oleic acid – C18:1(n-9)	0.28	2.37	1.15	1.11	1.12	
Docosanoic/behenic C22:0	0.15	0.2	0.19	0.15	0.14	
Hexadecenoic/palmitoleic C16:1(n-7)	0.22	0.23	0.23	0.23	0.23	
Linoleic acid – C18:2(n-6)	0.04	3.69	1.57	1.99	2.01	
γ-Linolenic acid – C18:3(n-6) (GLA)	0.1	0.09	0.1	0.09	0.09	
α-Linolenic acid – C18:3(n-3) (ALA)	0.16	0.19	0.18	0.18	0.18	
Arachidic acid – C20:0	0.21	0.24	0.23	0.12	0.23	
Eicosadienoic acid – C20:2(n-6)	< 0.01[a]	< 0.01[a]	< 0.01[a]	< 0.01[a]	< 0.01[a]	
Eicosatrienoic acid – C20:3(n-6)	0.26	0.23	0.25	0.23	0.24	
Arachidonic acid – C20:4(n-6)	0.14	0.14	0.15	0.15	0.15	
Eicosapentaenoic acid (EPA) – C20:5(n-3)	0.47	0.45	0.48	0.54	0.55	
Erucic acid – C22:1(n-9)	< 0.01[a]	< 0.01[a]	< 0.01[a]	< 0.01[a]	< 0.01[a]	
Docosapentaenoic acid– C22:5(n-3) (DPA n-3)	0.13	0.14	0.15	0.14	0.15	
Docosapentaenoic acid– C22:5(n-6) (DPA n-6)	14.51	13.11	14.22	12.98	13.15	
Docosahexaenoic acid (DHA) – C22:6(n-3)	56.44	53.34	56.07	59.59	61.16	
Lignoceric acid – C24:0	< 0.01[a]	< 0.01[a]	< 0.01[a]	0.22	0.22	
Trans fatty acids (g/100 g)	0.05	0.1	0.07	0.05	0.04	
Sterols (mg/100 g)						
Total sterols	444	260	389	328	316	NMKL 198:2014
Cholesterol	284	163	250	220	160	
Sitosterol	52	38	47	34	74	
Stigmasterol	22	13	19	16	16	
Campesterol	1	4	2	3	11	
Campestanol	3	1	2	< 1[a]	< 1[a]	
Brassicasterol	12	7	10	10	7	
Sitostanol+delta-5-avenasterol	18	5	14	6	6	
Delta-5, 24-stigmastadienol	15	4	12	8	6	
Delta-7-stigmastenol	32	23	29	28	31	
Delta-7-avenasterol	5	2	4	3	5	
Tocopherol (mg/100 g)						
α-Tocopherol	59.2	64.9	61.6	58.9	60.7	BS EN 12822:2014
β-Tocopherol	8.35	9.02	8.61	< 0.12[a]	< 0.12[a]	
γ-Tocopherol	285	299	300	334	334	
δ-Tocopherol	183	191	188	149	148	
α-Tocopherol equivalent	93.7	101	97.8	93.8	95.5	
Tocopherol (α + β + γ + δ)	536	565	558	542	524	

TABLE 1 (Continued)

Parameter (unit)	Batch number #1	#2	#3	#4	#5	Method of analysis
Metals						
Mercury (mg/kg)	< 0.005[a]	< 0.005[a]	< 0.005[a]	< 0.005[a]	< 0.005[a]	GB 5009.268-2016 I
Cadmium (mg/kg)	< 0.005[a]	< 0.005[a]	< 0.005[a]	< 0.005[a]	< 0.005[a]	
Arsenic (mg/kg)	< 0.005[a]	< 0.005[a]	< 0.005[a]	< 0.005[a]	< 0.005[a]	
Lead (mg/kg)	< 0.05[a]	< 0.05[a]	< 0.05[a]	< 0.05[a]	< 0.05[a]	
Copper (mg/kg)	< 0.05[a]	< 0.05[a]	< 0.05[a]	< 0.05[a]	< 0.05[a]	GB 5009.13-2017 method I
Iron (mg/100 g)	< 0.1[a]	< 0.1[a]	< 0.1[a]	< 0.1[a]	< 0.1[a]	AOAC 984.27, analysis was performed by ICP-OES.
Microbiological analysis						
Total Plate Count (CFU/g)	< 10[a]	< 10[a]	< 10[a]	< 10[a]	< 10[a]	ISO 4833-1:2013
Moulds (CFU/g)	< 10[a]	< 10[a]	< 10[a]	< 10[a]	< 10[a]	ISO 21527-1:2008
Yeast (CFU/g)	< 10[a]	< 10[a]	< 10[a]	< 10[a]	< 10[a]	ISO 21527-1:2008
Coliforms (MPN/g)	< 0.3[a]	< 0.3[a]	< 0.3[a]	< 0.3[a]	< 0.3[a]	ISO 4831:2006
Escherichia coli (/10 mL)	ND	ND	ND	ND	ND	ISO 7251:2005
Salmonella (/25 g)	ND	ND	ND	ND	ND	ISO 6579-1:2017
Coagulase-positive staphylococci (CFU/mL)	< 1[a]	< 1[a]	< 1[a]	< 1[a]	< 1[a]	ISO 6888-1:1999 Amd.2:2018(E)
Bacillus cereus (CFU/mL)	< 1[a]	< 1[a]	< 1[a]	< 1[a]	< 1[a]	ISO 7932:2004
Listeria monocytogenes (/25 mL)	ND	ND	ND	ND	ND	ISO 11290-1:2017
Enterobacteriaceae (/10 mL)	ND	ND	ND	ND	ND	ISO 21528-1:2017
Cronobacter sakazakii (/10 g)	ND	ND	ND	ND	ND	ISO 22964:2017
Contaminants						
Aflatoxin B1 (µg/kg)	< 0.3[a]	< 0.3[a]	< 0.3[a]	< 0.3[a]	< 0.3[a]	GB 5009.22-2016 III
Sum of free glycidol, glycidol-ester (determined as free glycidol) (µg/kg)	< 100[a]	< 100[a]	< 100[a]	< 100[a]	< 100[a]	AOCS Official Method cd 29b-13 GC–MS
Sum of free 3-MCPD and 3-MCPD esters (determined as free 3-MCPD (µg/kg)	200	230	280	200	230	AOCS Official Method cd 29b-13 GC–MS

Abbreviations: Abs, absent; AOAC, Association of Official Analytical Collaborations; AOCS, American Oil Chemists Society; CFU, Colony Forming Unit; FDA, United States Food and Drug Administration; GC–MS, Gas Chromatography with Mass Spectroscopy; ICP-OES, Inductively Coupled Plasma – Optical Emission Spectroscopy; ISO, International Organization for Standardization; MCPD, monochloro-propanol-1,2-diol; MPN, most probable number; ND, not detected.

[a]LOQ: limit of quantification.

[b]The fat content was analysed in five additional batches by using another method (GB 5009.6.2016 method II). Based on this method, the fat content in the NF was about 99.5–99.8 g/100 g.

[c]Value above the limit for *p*-anisidine in the specifications (≤ 10).

3.4.1 | Stability

3.4.1.1 | *Stability of the NF*

The applicant performed three stability tests with the pure algal oil (without the addition of sunflower oil): two batches were tested at −18°C up to 24 months and at 4°C up to 18 months; three batches were tested at 25 ± 2°C up to 4 months. The batches were regularly analysed for DHA content, peroxide and p-anisidine values. DHA contents and peroxide values complied with the proposed specifications at each time point tested (DHA content around 55%; peroxide values < 2 meq/g KOH). One of the two batches tested at −18°C and at 4°C showed the highest p-anisidine value (5.1 and 5.2, respectively) at 12 months. In the three batches tested at 25 ± 2°C, p-anisidine value increased from 8.4 ($t = 0$) to 9.8 ($t = 4$ month), from 6.3 ($t = 0$) to 8.4 ($t = 4$ month) and from 3.4 ($t = 0$) to 5.9 ($t = 4$ month), respectively.

Upon EFSA's request for information, the applicant provided three stability tests with several batches of algal oil added with sunflower oil and soybean-derived tocopherols: two batches were tested at −18°C for up to 24 months, two batches were tested at 4°C for up to 12 months and three batches were tested at 25 ± 2°C for up to 4 months.

The batches were regularly analysed for DHA content, peroxide and p-anisidine values. DHA contents and peroxide values complied with the proposed specifications (Table 2 – Section 3.5) at each time point tested in the three stability tests.

P-Anisidine value was up to 6.6 after 24 months at –18°C, up to 6.7 after 12 months at 4°C and up to 5.4 after 4 months at 25 ± 2°C.

Based on the stability tests the applicant recommends storing the NF at –18°C for 24 months and at 4°C for 12 months.

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

3.4.1.2 | *Stability of the NF under the intended conditions of use (addition to IF/FOF)*

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

During the assessment, EFSA requested information on the stability of the NF when undergoing powder processing. In reply, the applicant provided stability results on some batches of the NF in powder formulation: Four batches were tested at 65°C for up to 26 days and at 25°C for up to 26 months.

The batches were regularly analysed for DHA and free FA content, as well as peroxide and p-anisidine values. DHA, free FA contents and peroxide value complied with the proposed specifications at each time point tested in the two stability tests. The p-anisidine value was up to 9.01 after 26 days at 65°C and up to 4.01 after 26 months at 25°C.

Based on the stability tests provided on the NF and the powdered form of the NF, 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 (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, a maximum limit of 10 could be used for the *p*-anisidine value in *Schizochytrium* oils.

TABLE 2 Specifications of the NF.

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.

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

This genus has been used as a source of DHA-oils since 2003, the year of the first authorisation of DHA-oil from *Schizochytrium* sp. as NF. The first assessment of DHA-oil from *Schizochytrium* 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 '*Schizochytrium* 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: *Schizochytrium* sp. ATCC PTA-9695, *Schizochytrium* sp. T18, *Schizochytrium limacinum* WZU477 (EFSA NDA Panel et al., 2020) and *Schizochytrium limacinum* FCC-3204 (EFSA NDA Panel et al., 2021).

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, 11.08.2021, p. 24–27.

3.6.2 | History of use of the NF

In 2021, the applicant submitted a GRAS (Generally Recognised as Safe) notification to the Food and Drug Administration (FDA) for the DHA-oil from the microalgae *Schizochytrium* sp. (strain TKD-1) to be used in IF and in foods (GNR No 001008[4]). In 2022, 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. 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 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.

[4]https://www.fda.gov/media/156122/download

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 3 in Section 3.6.1). 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 ranged between 0.05 and 0.1 g/100 g, and based on the acid value, free FAs are not expected to be of concern. The FA profile reveals that DHA is the predominant compound. DHA is an essential nutrient for infants and children. 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 2600 and 4400 mg/kg and corresponds to 0.0026–0.0044 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 below the concentration of sterols reported in marketed IF and FOF (total animal sterols: 0.017–0.054 mg/mL; total plant sterols: 0.03–0.05 mg/mL reported by Claumarchirant et al., 2015; total sterols: 0.09–0.15 mg/mL reported by Hamdan et al., 2018).

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

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

The Panel considers that, taking into account the composition of the NF and the proposed conditions of use, the 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 TKD-1) 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 et al., 2020).

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.

No toxicity studies that were conducted with the NF under assessment (oil produced from strain TKD-1 from *Schizochytrium limacinum*) have been provided by the applicant.

However, the toxicity of DHA-rich algal oils produced from different strains of *Schizochytrium* sp. has been extensively investigated over the last decades. Several guideline compliant studies, including bacterial reverse mutation tests, in vitro chromosomal aberration tests, in vivo mammalian cell micronucleus tests, sub-chronic 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, Tran, Peach, Bauter, et al., 2012; Schmitt, Tran, Peach, Edwards, et al., 2012) 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 (Falk et al., 2017; Lewis et al., 2016), performed with DHA-oils from unspecified strains of *Schizochytrium* sp., have been considered in the assessment carried out by Anses (2018).

In all previous assessments, the competent authorities concluded that there were no concerns with regard to genotoxicity and subchronic toxicity of the tested materials. Further studies retrieved from the literature indicated the same outcome for a diversity of DHA-oils produced from other strains of *Schizochytrium* sp. (Abril et al., 2003; Blum et al., 2007; Hammond et al., 2001a, 2001b, 2002; Kroes 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, 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 Panel notes that tocopherols from soybean oil are added to the NF, potentially resulting in traces of soybean proteins. The compositional data on the NF indicate 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 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 microalgae *Schizochytrium* sp. (strain TKD-1). The available evidence indicates that the source organism (*Schizochytrium* sp., strain TKD-1) belongs to the species *Schizochytrium limacinum*. The source organism which is assessed in this application is *Schizochytrium limacinum* (strain TKD-1) 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', 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 53%–61% 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.

Toxicological tests with the NF were not performed. However, based on the available toxicological data of various forms of DHA-oils derived from *Schizochytrium* sp., the QPS status of the source of the NF (*Schizochytrium limacinum*), 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 TKD-1 belonging to species *Schizochytrium limacinum*, is safe under the proposed conditions of use. The target population is infants and young children.

6 | STEPS TAKEN BY EFSA

1. On 21/04/2021 EFSA received a letter from the European Commission with the request for a scientific opinion on the safety of *Schizochytrium* sp. (TKD-1) oil as a novel food. Ref. Ares(2021)2676891–21/04/2021.
2. On 21/04/2021, a valid application on *Schizochytrium* sp. (TKD-1) oil, which was submitted by TK Biohealth Co., LTD. (China), was made available to EFSA by the European Commission through the Commission e-submission portal (NF 2020/2242) and the scientific evaluation procedure was initiated.
3. On 23/07/2021, 14/12/2021 and 18/11/2022, EFSA requested the applicant to provide additional information to accompany the application and the scientific evaluation was suspended.
4. On 01/11/2021, 08/09/2022 and 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 limacinum* (strain TKD-1) 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
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
bp	base pair
bw	body weight
CONTAM	EFSA Panel on Contaminants
DHA	docosahexaenoic acid
DPA	docosapentaenoic acid
EPA	eicosapentaenoic acid
FA	fatty acids
FAIM	Food Additive Intake Model
FDA	Food and Drug Administration
FOF	follow-on formula
FSAI	Food Safety Authority of Ireland
GRAS	Generally Recognized as Safe
HACCP	Hazard Analysis Critical Control Points
IF	infant formula
LOQ	limit of quantification
MCPD	Monochloro-Propanol-1,2-Diol
NDA	EFSA Panel on Nutrition, Novel Foods and Food Allergens
NF	novel food
PUFA	polyunsaturated fatty acids
QPS	Qualified presumption of safety
rRNA	ribosomal Ribonucleic Acid
UK	United Kingdom

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

EFSA-Q-2020-00850

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REFERENCES

Abril, R., Garrett, J., Zeller, S. G., Sander, W. J., & Mast, R. W. (2003). Safety assessment of DHA-rich microalgae from *Schizochytrium* sp. – Part V: Target animal safety/toxicity study in growing swine. *Regulatory Toxicology and Pharmacology*, *37*, 73–82.

Anses [Agence nationale de securit e sanitaire de l 'alimentation, de l'environnement et du travail (French Agency for Food, Environmental and Occupational Health and Safety)]. (2018). Avis de l'Agence nationale de securit e alimentaire de l'alimentation, de l'environnement et du travail relatif a une demande d 'autorisation de mise sur le marche d 'un nouvel aliment ou ingredient alimentaire: huile riche en DHA issue du *Schizochytrium* sp. Mai, 2018.

Blum, R., Kiy, T., Waalkens-Berendsen, I., Wong, A. W., & Roberts, A. (2007). One-generation reproductive toxicity study of DHA-rich oil in rats. *Regulatory Toxicology and Pharmacology*, *49*, 260–270.

Claumarchirant, L., Matencio, E., Sanchez-Siles, L. M., Amparo Alegrıa, A., & Lagarda, M. J. (2015). Sterol composition in infant formulas and estimated intake. *Journal of Agricultural and Food Chemistry*, *63*, 7245–7251.

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Koutsoumanis, K., Allende, A., Alvarez-Ordonez, A., Bolton, D., Bover-Cid, S., Chemaly, M., Davies, R., De Cesare, A., Hilbert, F., Lindqvist, R., Nauta, M., Peixe, L., Ru, G., Simmons, M., Skandamis, P., Suffredini, E., Cocconcelli, P. S., Fernandez Escamez, P. S., … Herman, L. (2020). Statement on the update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA: Suitability of taxonomic units notified to EFSA until September 2019. *EFSA Journal*, *18*(2), 5965. https://doi.org/10.2903/j.efsa.2020.5965

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Koutsoumanis, K., Allende, A., Alvarez-Ordonez, A., Bolton, D., Bover-Cid, S., Chemaly, M., De Cesare, A., Hilbert, F., Lindqvist, R., Nauta, M., Peixe, L., Ru, G., Simmons, M., Skandamis, P., Suffredini, E., Cocconcelli, P. S., Fernández Escámez, P. S., Prieto Maradona, M., … Herman, L. (2023). *Updated list of QPS-recommended microorganisms for safety risk assessments carried out by EFSA*. https://zenodo.org/record/7554079

EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain). (2009). Scientific opinion of the panel on contaminants in the food chain on a request from the European Commission on marine biotoxins in shellfish – Summary on regulated marine biotoxins. *EFSA Journal*, *7*(8), 1306. https://doi.org/10.2903/j.efsa.2009.1306

EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies). (2010). Scientific opinion on dietary reference values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, trans fatty acids, and cholesterol. *EFSA Journal*, *8*(3), 1461. https://doi.org/10.2903/j.efsa.2010.1461

EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies). (2012). Scientific opinion related to the tolerable upper intake level of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and docosapentaenoic acid (DPA). *EFSA Journal*, *10*(7), 2815. https://doi.org/10.2903/j.efsa.2012.2815

EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies). (2013). Scientific opinion on nutrient requirements and dietary intakes of and young children the European Union. *EFSA Journal*, *11*(10), 3408. https://doi.org/10.2903/j.efsa.2013.3408

EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies). (2014). Scientific opinion on the essential composition of infant and follow-on formulae. *EFSA Journal*, *12*(7), 3760. https://doi.org/10.2903/j.efsa.2014.3760

EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies). (2016). Guidance on the preparation and presentation of an application for authorisation of a novel food in the context of Regulation (EU) 2015/2283. *EFSA Journal*, *14*(11), 4594. https://doi.org/10.2903/j.efsa.2016.4594

EFSA NDA Panel (EFSA Panel on Nutrition, Novel Foods and Food Allergens), Turck, D., Castenmiller, J., De Henauw, S., Hirsch-Ernst, K. I., Kearney, J., Maciuk, A., Mangelsdorf, I., McArdle, H. J., Naska, A., Pelaez, C., Pentieva, K., Siani, A., Thies, F., Tsabouri, S., Vinceti, M., Cubadda, F., Frenzel, T., Heinonen, M., … Knutsen, H. K. (2021). Scientific opinion on the safety of oil from *Schizochytrium limacinum* (strain FCC-3204) for use in infant and follow-on formula as a novel food pursuant to Regulation (EU) 2015/2283. *EFSA Journal*, *19*(1), 6344.

EFSA NDA Panel (Panel on Nutrition, Novel Foods and Food Allergens), Turck, D., Castenmiller, J., De Henauw, S., Hirsch-Ernst, K. I., Kearney, J., Maciuk, A., Mangelsdorf, I., McArdle, H. J., Naska, A., Pelaez, C., Pentieva, K., Siani, A., Thies, F., Tsabouri, S., Vinceti, M., Cubadda, F., Engel, K. H., Frenzel, T., … Knutsen, H. K. (2020). Scientific opinion on the safety of *Schizochytrium* sp. oil as a novel food pursuant to regulation (EU) 2015/2283. *EFSA Journal*, *18*(10), 6242.

European Pharmacopoeia, 11.0th Edition. (2014). Monograph on salmon oil, farmed, pp. 3941–3943.

Falk, M. C., Zheng, X., Chen, D., Jiang, Y., Liu, Z., & Lewis, K. D. (2017). Developmental and reproductive toxicological evaluation of arachidonic acid (ARA)-rich oil and docosahexaenoic acid (DHA)-rich oil. *Food and Chemical Toxicology*, *103*, 270–278.

Fedorova-Dahms, I., Marone, P. A., Bauter, M., & Ryan, A. S. (2011). Safety evaluation of DHA-rich algal oil from *Schizochytrium* sp. *Food and Chemical Toxicology*, *49*, 70–77.

FSAI (Food Safety Authority of Ireland). (2014). Substantial Equivalence Opinion DHA-Rich Algal Oil. Available online: https://www.fsai.ie/uploadedFiles/Science_and_Health/Novel_Foods/Notifications/Fermentalg%20DHA%20rich%20oil.pdf

Hamdan, I. J. A., Sanchez-Siles, L. M., Garcia-Llatas, G., & Lagarda, M. J. (2018). Sterols in infant formulas: A bioaccessibility study. *Journal of Agricultural and Food Chemistry*, *66*, 1377–1385.

Hammond, B. G., Mayhew, D. A., Kier, L. D., Mast, R. W., & Sander, W. J. (2002). Safety assessment of DHA-rich microalgae from *Schizochytrium* sp. – IV. Mutagenicity studies. *Regulatory Toxicology and Pharmacology*, *35*, 255–265.

Hammond, B. G., Mayhew, D. A., Naylor, M. W., Ruecker, F. A., Mast, R. W., & Sander, W. J. (2001a). Safety assessment of DHArich microalgae from *Schizochytrium* sp. – I. Subchronic rat feeding study. *Regulatory Toxicology and Pharmacology*, *33*, 192–204.

Hammond, B. G., Mayhew, D. A., Naylor, M. W., Ruecker, F. A., Mast, R. W., & Sander, W. J. (2001b). Safety assessment of DHA rich microalgae from *Schizochytrium* sp. – II. Developmental toxicity evaluation in rats and rabbits. *Regulatory Toxicology and Pharmacology*, *33*, 205–217.

Kanner, J. (2007). Dietary advanced lipid oxidation endproducts are risk factors to human health. *Molecular Nutritional Food and Research*, *51*, 1094–1101.

Kroes, R., Schaefer, E. J., Squire, R. A., & Williams, G. M. (2003). A review of the safety of DHA45-oil. *Food and Chemical Toxicology*, *41*, 1433–1446.

Lewis, K. D., Huang, W., Zheng, X., Jiang, Y., & Feldman, R. S. (2016). Toxicological evaluation of arachidonic acid (ARA)-rich oil and docosahexaenoic acid (DHA)-rich oil. *Food and Chemical Toxicology*, *96*, 133–144.

Schmitt, D., Tran, N., Peach, J., Bauter, M., & Marone, P. (2012). Toxicologic evaluation of DHA-rich algal oil: Genotoxicity, acute and subchronic toxicity in rats. *Food and Chemical Toxicology*, *50*, 3567–3676.

Schmitt, D., Tran, N., Peach, J., Edwards, T., & Greeley, M. (2012). Toxicologic evaluation of DHA-rich algal oil in rats: Developmental toxicity study and 3-month dietary toxicity study with an in utero exposure phase. *Food and Chemical Toxicology*, *50*, 4149–4157.

United Kingdom. (2002). *Opinion on an application under the Novel Food Regulation from OmegaTech for clearanceof DHA GoldTM, a DHA rich oil*. https://webarchive.nationalarchives.gov.uk/20200405232644/https://acnfp.food.gov.uk/assess/fullapplics/60694

United Kingdom. (2014). *Advisory Committee on Novel Foods and Processes. Opinion on an application under the Novel Food Regulation for DHA rich oil from the microalgae Schizochytrium*. https://acnfp.food.gov.uk/sites/default/files/dha-initial-opinion-letter.pdf

United Kingdom. (2017). *Advisory Committee on Novel Foods and Processes. Opinion on an application under the Novel Food Regulation for DHA rich algal oil from Schizochytrium species T18*. https://acnfp.food.gov.uk/sites/default/files/dhamara_1_0.pdf

Vieira, S. A., Zhang, G. & Decker, E. A. (2017). Biological implications of lipid oxidation products. *Journal of the American Oil Chemists Society*, *94*, 339–351.

Yokoyama, R., & Honda, D. (2007). Taxonomic rearrangement of the genus *Schizochytrium* sensu lato based on morphology, chemotaxonomical characteristics and 18S rRNA gene phylogeny (Thraustochytriaceae, Labyrinthulomycetes): Emendation for *Schizochytrium* and erection of Aurantiochytrium and Oblongichytrium gen. nov. *Mycoscience*, *48*, 199–211.

How to cite this article: EFSA NDA Panel (EFSA Panel on Nutrition, Novel Foods and Food Allergens), Turck, D., Bohn, T., Castenmiller, J., De Henauw, S., Hirsch-Ernst, K. I., Maciuk, A., Mangelsdorf, I., McArdle, H. J., Naska, A., Pelaez, C., Pentieva, K., Siani, A., Thies, F., Tsabouri, S., Vinceti, M., Aguilera-Gómez, M., Cubadda, F., … Knutsen, H. K. (2023). Safety of oil from *Schizochytrium limacinum* (strain TKD-1) for use in infant and follow-on formula as a novel food pursuant to Regulation (EU) 2015/2283. *EFSA Journal*, *21*(12), e8414. https://doi.org/10.2903/j.efsa.2023.8414