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Safety of an ethanolic extract of the dried biomass of the microalga *Phaeodactylum tricornutum* 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 an ethanolic extract of the dried biomass of the microalga *Phaeodactylum tricornutum* as a novel food (NF) pursuant to Regulation (EU) 2015/2283. The NF is an ethanolic extract of the dried biomass of the microalga *P. tricornutum* diluted in a medium-chain triglyceride oil carrier, with standardised fucoxanthin and tocopherol content. The main component of the NF is fat (78% on average), followed by crude protein (10% on average). The Panel is of the view that a consistent and safe production process has not been demonstrated. Additionally, the Panel considers that the information provided on the composition of the NF is not complete and may raise safety concerns. The applicant proposed to use the NF as a food supplement at the use level of 437 mg/day, with the target population being adults, excluding pregnant and breastfeeding women. There is no history of use of the NF or of its source, i.e. *P. tricornutum*. The Panel notes that the source of the NF, *P. tricornutum*, was not granted the qualified presumption of safety (QPS) status by the EFSA Panel on Biological Hazards (BIOHAZ), due to the lack of a safe history of use in the food chain and on its potential for production of bioactive compounds with toxic effects. There were no concerns regarding genotoxicity of the NF. In the 90-day study provided, a number of adverse effects were observed, some of them seen already at the lowest dose tested (750 mg/kg body weight (bw) day), which was identified by the Panel as the lowest-observed-adverse-effect-level (LOAEL). The potential phototoxicity of pheophorbide A and pyropheophorbide A in the NF was not addressed in this study. Although noting the uncertainties identified by the Panel regarding the analytical determination of these substances in the NF and the limitations in the publicly available toxicity data, a low margin of exposure (MoE) was calculated for these substances at the proposed use levels. The Panel concludes that the safety of the NF under the proposed uses and use levels has not been established.

Keywords: novel foods, food safety, food supplement, *Phaeodactylum tricornutum*, microalgae, lipids, fucoxanthin

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1. Introduction

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

On 18 December 2018, the company Microphyt submitted a request to the European Commission in accordance with Article 10 of Regulation (EU) No 2015/2283 to place the extract of the microalga *Phaeodactylum tricornutum* on the Union market as an NF.

The novel food (extract of *Phaeodactylum tricornutum*) is intended to be used as an ingredient in food supplements. The target population is the general adult population.[1]

On 15 May 2019, in accordance with Article 10(3) of Regulation (EU) 2015/2283, the European Commission asked the European Food Safety Authority to provide a scientific opinion by carrying out the assessment for the extract of the microalga *Phaeodactylum tricornutum* as a novel food.

1.2. Additional information

In 2014, the Joint Research Centre of the European Commission published the scientific and policy report 'Microalgae-based products for the food and feed sector: an outlook for Europe', in which *Phaeodactylum tricornutum* was listed among the algal species without known toxin production.

In 2019, *P. tricornutum* was assessed by the EFSA BIOHAZ Panel for its suitability to be added to the list of qualified presumption of safety (QPS)-recommended biological agents intentionally added to food or feed (EFSA BIOHAZ Panel, 2019). The BIOHAZ Panel summarised those newer studies that linked *P. tricornutum* in the presence of β-*N*-methylamino-L-alanine (BMAA) (Réveillon et al., 2016a; Lage et al., 2019), a neurotoxin produced by certain cyanobacteria, diatoms, and dinoflagellates. Due to the lack of a safe history of use in the food chain and its potential for the production of bioactive compounds with toxic effects, the BIOHAZ Panel concluded that *P. tricornutum* cannot be granted the QPS status.

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, and additional data identified by the Panel.

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

A common and structured format for the presentation of NF applications is described in the EFSA guidance on the preparation and presentation of an 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 includes a request for protection of proprietary data in accordance with Article 26 of Regulation (EU) 2015/2283. The data requested by the applicant to be protected comprise information on the identification *of P. tricornutum* by molecular techniques and on the confidential deposit of the *P. tricornutum* in the Culture Collection of Algae and Protozoa (CCAP), the detailed production process, development, and validation of analytical methods, compositional data, specifications, nutritional information, toxicological studies (90-day repeated dose oral toxicity, genotoxicity studies) and information on allergenicity.

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] Excluding pregnant and breastfeeding women.

[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.

Additional information that was not included in the application was retrieved by literature search following a search strategy and standard operating procedure as described by Dibusz and Vejvodova (2020).

This assessment concerns only the risks that might be associated with the 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 ethanolic extract of the microalga *Phaeodactylum tricornutum* dried biomass in a medium-chain triglyceride (MCT) oil carrier. The NF falls under the category 'food consisting of, isolated from or produced with microorganisms, fungi or algae', as described in Article 3 of Regulation (EU) 2015/2283. Fucoxanthin (FX) and tocopherol contents are standardised. The NF is proposed to be used as an ingredient in food supplements, with the target population being the general adult population, excluding pregnant and breastfeeding women.

3.2. Identity of the NF

The NF is an ethanolic extract of *P. tricornutum*'s dried biomass diluted in an MCT-oil carrier, with standardised FX and tocopherol content. The NF consists mainly of fat, with triglycerides being the main lipid class. The source of the NF, *P. tricornutum*, a microalga belonging to the family of Phaeodactylaceae, has never been used before for human consumption. *P. tricornutum* Bohlin, 1897 is the scientific name originally given to this species. The strain used to produce the NF was identified by molecular techniques using 25S rRNA and housekeeping gene markers (strain number Mi136 M1a). The identification was conducted by the external testing facility IDmyk Eurofins. Internal databases (i.e. Fungi V30 EIDmyk) and NCBI BLAST complementary analysis were used for identification. The highest homology of the strain has been found to exist with the following reference strains: *P. tricornutum* RCC69 18S (100%), *P. tricornutum* CCMP2561 (100%), *P. tricornutum* CCMP631 (99%), *P. tricornutum* CCMP630 (99%), *P. tricornutum* CCAP1052/6 (99%), *P. tricornutum* CCMP 632 5 (99%) and *P. tricornutum* CCMP1327 (99%). The wild-type strain of *P. tricornutum* was isolated from one of the applicant's cultures and has been deposited on 14 May 2018 in CCAP, Scotland, United Kingdom (Gachon et al., 2007).

3.3. Production process

According to the information provided, the NF is produced in line with Hazard Analysis Critical Control Points (HACCP) principles. The applicant stated that Good Manufacturing Practice (GMP) is followed but the facility does not hold an official GMP certification. The microalga is cultivated under controlled conditions.

The above-described strain of *P. tricornutum* is the starting biological material of the production process. The strain is propagated and cultivated in an artificial marine culture medium produced by the applicant, under controlled conditions (light intensity, photoperiod, temperature, gas flow rate, pH). Qualitative and quantitative information on the constituents of the culture medium has been provided by the applicant. In addition, upon inoculation and alongside the cultivation process, vitamins may be added to the culture medium. The water used in the production process is obtained by reverse osmosis and is regularly controlled regarding the levels of heavy metals, pesticides, and microbiological agents. When a certain density of the culture is reached, the harvest of the microalgal biomass is carried out, under a semi-continuous mode. Cytomorphology and organoleptic characteristics of the harvested biomass are controlled, and the harvested biomass is stored for up to 24 h, in the dark, without thermal regulation.

The liquid culture medium is separated from the harvested biomass by centrifugation, and the resulting intermediate product, with ca. 20% w/w dry matter, is stored at –18°C. The frozen biomass is then thawed and undergoes oven-drying under vacuum (45–60°C), to reach a water content of less than 10% w/w. The dried biomass is subsequently ground and stored at 5°C until extraction. The extraction phase includes extraction with absolute ethanol[3] under controlled temperature and mixing

[3] From the information provided, it could not be concluded whether the absolute ethanol (≥ 99.9%vol) used is appropriate for food production.

conditions, filtration under vacuum assisted by diatomaceous earth, and evaporation of the solvent under controlled temperature and pressure conditions. MCT oil, based on coconut oil, is added to the concentrated extract, simultaneously with to the evaporation step, to facilitate solvent evaporation and to standardise the FX levels (2% w/w). A tocopherol-rich extract (E 306) is added (0.5% w/w) to enhance the NF's oxidative stability. The NF is stored in dedicated containers, under nitrogen, at 5°C.

The applicant investigated and demonstrated the absence of viable *P. tricornutum* cells, in the dried and ground biomass, as well as in the NF.

The Panel considers that the production process is sufficiently described.

3.4. Compositional data

In order to confirm that the production process is reproducible and adequate to produce on a commercial scale a product with certain characteristics, the applicant provided qualitative and quantitative data of chemical and microbiological parameters for a number of different batches of the NF (five or six) independently produced. The NF has a liquid appearance, and is of green-liquid colour, with a characteristic odour. As indicated from the results of the proximate analysis (Table 1), the NF has, on average, a fat content of 77.8 ± 1.8% w/w, crude protein 8.9 ± 0.6% w/w, total carbohydrates 10.1 ± 0.9% w/w and ash 2.1 ± 0.4% w/w.

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

Parameter (unit)	Batch number					
	#1	#2	#3	#4	#5	Analytical method
Moisture (g/100 g of NF)	2.59	2.70	0.15	0.3	0.38	ASU L 17.00–1 mod.
Fat (g/100 g of NF)	75.90	75.75	79.55	78.63	79.08	ASU L 06.00–6 (Weibull-Stoldt)
Saturated fatty acids (g/100 g of NF)	68	68	68	70	70	GC-FID
Monounsaturated fatty acids (g/100 g of NF)	3.0	3.0	6.1	3.5	3.3	GC-FID
Polyunsaturated fatty acids (g/100 g of NF)	5.2	4.9	5.8	5.5	6.2	GC-FID
Crude protein (g/100 g of NF)	8.4	8.3	9.61	9.23	9.14	ASU L 06.00–20, Dumas
Ash (g/100 g of NF)	2.56	2.13	2.17	1.82	1.68	ASU L 06.00–4 mod.
Total carbohydrates (g/100 g of NF)	10.5	11.1	8.7	10.3	10.1	Calculation[(a)]
Total sugars (g/100 g of NF)	0.27	0.23	ND	ND	ND	Calculation[(b)]
Energy (kcal/100 g of NF)	758.9	759.4	789.1	785.9	788.7	Regulation (EU) 1169/2011

NF: novel food; ND: not detected; ASU: Amtliche Sammlung von Untersuchungsverfahren (Official Collection of Methods of Analysis); GC-FID: gas chromatography-flame ionisation detection.
(a): Total carbohydrates = 100 – (moisture + fat + crude protein +ash).
(b): Total sugars = D-Glucose + Fructose + Sucrose + Lactose + Maltose (determined enzymatically).

The maximum peroxide value of the NF has been reported to be 0.3 meq $O_2.kg^{-1}$ among the batches analysed. Additionally, the applicant provided qualitative and quantitative data on the lipid profile of the NF (triglycerides, diglycerides, monoglycerides, free fatty acids (FFAs), sterols, phospholipids). The most prevalent lipid class is triglycerides (ca. 80.76 ± 4.50% of total fat). The Panel notes the levels of total FFAs reported by the applicant (13.12 ± 4.77% of total fat). High levels of FFAs in oils may result due to lipases, due to processing (i.e. high temperature and relative humidity, tissue damage), or storage conditions (Balduyck et al., 2016; Ryckebosch et al., 2011). Moreover, the Panel notes that phospholipids (4.13 ± 1.17% the NF), due to their nature, can interfere with the analytical method used for the determination of acid value/FFA, leading thus, to overestimation of the results. Ryckebosch et al. (2013, 2014) reported that oil extracted from *P. tricornutum* contains high levels of phospholipids (28.85–30.1% of total fat). The NF contains sterols representing (ca. 0.74 ± 0.38% of fat) (Table 2), with brassicasterol being the main one (93.54 ± 2.07% of total sterols). The fatty acid (FA) profile of the NF was determined in the same batches of the NF, which is discussed in Section '3.9 Nutritional information'. Qualitative and quantitative data on the levels of squalene, carotenoids, chlorophylls, and derivatives of chlorophylls, in the NF were also provided). FX is a carotenoid that occurs naturally in *P. tricornutum*

(Kim et al., 2012). On average, the NF contains 2.06% w/w FX, of which ca. 90% is all-*trans*-FX.[4] The all-*trans*-form of FX has been reported to be the major carotenoid of *P. tricornutum* (Kim et al., 2012; Kawee-ai et al., 2013). Substantially lower amounts of other carotenoids have been found in the NF, compared to FX levels.

Table 2: Batch-to-batch analysis of physicochemical properties, lipids, and liposoluble compounds of the NF

Parameter (unit)	Batch number					Analytical methods
	#6	#7	#8	#9	#10	
Peroxide value ($meqO_2.kg^{-1}$)	0.2	0.3	0.1	0.1	0.1	Potentiometry (NF EN ISO 27107)
Lipids (Relative % w/w of fat)						
Triglycerides	82	81	81.7	85.7	73.4	Gas chromatography (internal)
Diglycerides	3.5	4.5	4.3	2.8	3	Gas chromatography (internal)
Monoglycerides	1.6	1.5	1.2	1.2	1.3	Gas chromatography (internal)
Total sterols	0.3	1.3	0.8	0.5	0.8	NF EN ISO 12228_part 1
Cholesterol	< 0.1	0.1	0.4	< 0.1	< 0.1	Gas chromatography (internal)
Unidentified	0.6	0.9	< 0.1	< 0.1	< 0.1	Calculation
Free fatty acids (FFAs)	12	10.6	11.7	9.8	21.5	Gas chromatography (internal)
Total phospholipids (g/100 g of NF)	5.2	2.26	5.04	4.19	3.96	HPLC-ELSD (internal)
Total fucoxanthin (% w/w of NF)	2.06	2.05	2.01	2.11	2.09	HPLC-DAD (internal)
Chlorophylls and derivatives						
Chlorophyll C (g equivalent chlorophyll A /100 g of NF)	2.93	3.73	3.52	4.15	3.37	HPLC-DAD (internal)
Pheophorbide A (g equivalent pheophorbide A /100 g of NF)	1.88	1.59	3.35	2.01	2.15	HPLC-DAD (internal)
Pheophytin A (g equivalent chlorophyll A /100 g of NF)	ND(a)	0.56	0.671	0.36	0.49	HPLC-DAD (internal)
Σ Chlorophyll B (g equivalent chlorophyll A /100 g of NF)	ND(a)	ND(a)	ND(a)	ND(a)	ND(a)	HPLC-DAD (internal)
Σ Chlorophyll A (g equivalent chlorophyll A /100 g of NF)	ND(a)	0.651	0.031	0.169	0.122	HPLC-DAD (internal)

NF: novel food; ND: not detected; HPLC-DAD: high-performance liquid chromatography–diode-array detection; EN: Europaische Norm (European Standard); ISO: International Organization for Standardization; FFA: free fatty acid; HPLC-ELSD: high-performance liquid chromatography–evaporative light scattering detection.

(a): The applicant informed EFSA that the limits of quantification were not determined because the contents found in the samples were well above the minimum quantities injected during calibration.

Upon EFSA's request, the applicant investigated the levels of *trans*-FAs in the NF (Table 3). The sample preparation was done according to NF EN ISO 12966-2 and the analysis according to NF EN ISO 12966-4 (gas chromatography of FA methyl esters –capillary gas chromatography). In all the analysed NF batches, *trans* FAs were below the limit of quantification (LOQ) of the implemented analytical method.

Table 3: Batch-to-batch analysis of *trans* fatty acids in the NF

***Trans* fatty acid (% of the NF)**	**#11**	**#12**	**#13**	**#14**	**#15**
18:1 *trans*	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
18:2 *trans*	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
18:3 *trans*	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Total *trans*	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05

[4] On top of the fucoxanthin results presented in Table 2, the applicant provided additional analytical results on fucoxanthin concentrations, in the same NF batches but with the analysis performed by another laboratory implementing the same analytical method. The results were in a similar range (2.16–2.28%).

The Panel notes that pheophorbide A, a product of chlorophyll's breakdown, was detected in all the NF batches analysed. Hwang et al. (2005) reported that a high content of pyropheophorbide A (PPHA), alongside pheophorbide A (PHA), were responsible for phototoxicity induced by batches of the dried purple laver product 'nori'. Additionally, an animal study by Endo et al. (1982) showed that PPHA induced phototoxicity at lower doses compared to PHA. Considering the toxicological concern and the fact that in the study of Hwang et al. (2005) PPHA was quantified in higher concentrations than PHA in the dried purple laver product the applicant was requested to investigate the levels of PPHA in the NF.

Initially, the applicant attempted to investigate the presence of PPHA in the NF, using an internal high-performance liquid chromatography with ultraviolet detection (HPLC–UV) method and PPHA methyl ester as a standard. However, EFSA noted that the analytical method implemented was not adequate to separate PHA from PPHA. The applicant (using an external analytical laboratory) developed an HPLC–MS method using PPHA as standard and analysed five independently produced batches of the NF. The analysis revealed PPHA levels ranging from 0.2 up to 2.68 g/kg (i.e. 200–2,680 μg/g). Based on the provided body of evidence, the Panel is of the view that uncertainties remain regarding the validation of the analytical method implemented for the detection of PPHA. The Panel notes that the presence of PPHA has been reported in the literature in chlorella, spirulina, jews mallow, aloe, kale, green leaves (Oshima et al., 2004), as well as in salted vegetables (Takeda et al., 1985). In both studies, the concentrations reported were considerably lower compared to the NF (up to 24 μg/g and up to 387.2 μg/g, respectively).

The applicant conducted analyses to determine the levels of various phycotoxins (Table 4), neurotoxins (Table 5), heavy metals, dioxins and polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), residual solvents and mycotoxins in the NF (Table 6).

Table 4: Batch-to-batch analysis of phycotoxins in the NF

Parameter (unit)	Batch number					Analytical methods
	#6	#7	#8	#9	#10	
Domoic acid (mg/kg of NF)	< 0.3	< 0.3	1.4	1.4	1.6	LNRBM-ASP01 (HPLC/UV)
Okadaic acid, free (μg eqOA/kg of NF)	< 5	< 5	< 5	< 5	< 5	ANSES PBM BM LSA-INS-0147 (LC/MS–MS)
Okadaic acid, total (μg eqOA/kg of NF)	< 5	< 5	< 5	< 5	< 5	
Dinophysistoxin 1, free (μg eqOA/kg of NF)	< 5	< 5	< 5	< 5	< 5	
Dinophysistoxin 1, total (μg eqOA/kg of NF)	< 5	< 5	< 5	< 5	< 5	
Dinophysistoxin 2, free (μg eqOA/kg of NF)	< 3	< 3	< 3	< 3	< 3	
Dinophysistoxin 2, total (μg eqOA/kg of NF)	< 3	< 3	< 3	< 3	< 3	
Pectenotoxin 1 (μg eqPTX2/kg of NF)	< 2	< 2	< 2	< 2	< 2	
Pectenotoxin 2 (μg eqPTX2/kg of NF)	< 2	< 2	< 2	< 2	< 2	
Azaspiracid 1 (μg eqAZA1/kg of NF)	< 2	< 2	< 2	< 2	< 2	
Azaspiracid 2 (μg eqAZA1/kg of NF)	< 4	< 4	< 4	< 4	< 4	
Azaspiracid 3 (μg eqAZA1/kg of NF)	< 3	< 3	< 3	< 3	< 3	
SUM AZAs (μg eqAZA1/kg of NF)	< 4	< 4	< 4	< 4	< 4	
Yessotoxins (μg eqYTX/kg of NF)	< 5	< 5	< 5	< 5	< 5	
SUM YTXs (μg eqYTX/kg of NF)	< 5	< 5	< 5	< 5	< 5	
Gymnodimine (μg eqGYM/ kg of NF)	< 0.4	< 0 .4	< 0.4	<0.4	< 0.4	
Spirolide A (μg eqSPX1/kg of NF)	< 2	< 2	< 2	< 2	< 2	
Spirolide B (μg eqSPX1/kg of NF)	< 2	< 2	< 2	< 2	< 2	
Spirolide C (μg eqSPX1/kg of NF)	< 2	< 2	< 2	< 2	< 2	
Spirolide D (μg eqSPX1/kg of NF)	< 2	< 2	< 2	< 2	< 2	

NF: novel food; LC–MS/MS: liquid chromatography–tandem mass spectrometry; ANSES: Administración Nacional de la Seguridad Social (National Social Security Administration, Spain).

The results showed that all the phycotoxins tested, apart from domoic acid, are below the limits of detection (LODs) of the implemented methods. The maximum concentration of domoic acid reported in the NF (1.8 mg/kg of NF) is considerably lower compared to the concentrations of domoic acid reported in shellfish (EFSA, 2009).

Since the putative production of BMAA and 2,4-diaminobutyric acid (DAB) by *P. tricornutum* has been reported in literature (Réveillon et al., 2016a,b; Lage et al., 2019), quantitative analytical data on BMAA and its isomers have been provided by the applicant upon EFSA's request. The levels of free BMAA and its isomer DAB, were found to be below the respective LODs, in all but one of the NF batches tested (Table 5).

Table 5: Batch-to-batch analysis of BMAA and DAB in the NF

Parameter (unit)	Batch number					Analytical method
	#2	#16	#17	#3	#5	
Free BMAA (μg/g)	< LOD[(a)]	< LOD[(a)]	< LOD[(a)]	< LOD[(a)]	<LOD[(a)]	HILIC-MS/MS
Free DAB (μg/g)	< LOD[(a)]	< LOD[(a)]	< LOD[(a)]	< LOD[(a)]	0.41	

NF: novel food; HILIC–MS/MS: hydrophilic interaction chromatography–tandem mass spectroscopy; BMAA: β-*N*-methylamino-L-alanine; DAB: 2,4-diaminobutyric acid.
(a): LOD = 0.15 μg/g of dry weight corresponding to LOD = 0.26–0.33 μg/g of NF.

The Panel notes that the LOD of the implemented HILIC–MS/MS method was reported to be 0.15 μg/g of dry matter of the NF. Upon EFSA's request, the applicant reported that, considering the production process, the LOD of 0.15 μg/g in the dry matter of the NF would correspond to 0.26–0.33 μg/g of the NF. The applicant also provided EFSA literature data on the occurrence of BMAA and DAB in other foods (further described in Section 3.7.3. Estimated exposure to undesirable substances).

Additionally, the applicant provided analytical data on anatoxin-a, saxitoxin, and cylindrospermopsin in an intermediate product of the NF production (dried biomass), instead of the NF per se, arguing that the analysis was not feasible on an oily matrix (i.e. the NF). The reported values were below the LODs of the analytical in-house method used (HPLC–MS/MS). The Panel notes that the applicant did not manage to provide the requested documentation of the in-house method used (HPLC–MS/MS) to investigate the presence of anatoxin-a, saxitoxin, and cylindrospermopsin in the intermediate product, thus such data cannot be used to conclude on the presence of these toxins. The applicant argues that the three neurotoxins anatoxin-a, saxitoxin and cylindrospermopsin are produced by cyanobacteria, and, in the case of saxitoxin, by dinoflagellates too, and that it is unlikely to be produced by *P. tricornutum,* which is a diatom. Considering that these compounds can be produced by organisms that can contaminate the cultivation system, the applicant elaborated further on the species and presence/absence of organisms with the potential to produce these toxins in a microalgae cultivation system. The applicant attempted to investigate the presence of cyanobacteria, in triplicates, at the three stages of the production process (laboratory scale, bioreactor of 500 L, bioreactor of 5000 L). The tests were performed using a ready-to-use kit. The cyanobacteria concentration was assessed by microscopy (samples 'surface water'). In the specific nine samples analysed, no cyanobacteria were detected. Moreover, information on the measures taken to mitigate the presence of these organisms was provided by the applicant.

Considering the applicant's arguments above regarding the absence of cyanobacteria, the source of DAB in the NF cannot be determined with certainty.

Table 6: Batch-to-batch analysis of chemical contaminants in the NF

Parameter (unit)	Batch number					Analytical methods
	#6	#7	#8	#9	#10	
Heavy metals (mg/kg of NF)						
Lead	< 0.02	< 0.02	< 0.02	0.02	< 0.02	Mineralisation: EN 13805, mod. ICP-MS, EN 15763 mod.
Cadmium	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	
Arsenic	< 0.05	< 0.05	0.08	< 0.05	0.05	
Mercury	< 0.005	< 0.005	< 0.005	< 0.005	0.006	
Tin	< 1	< 1	< 1	< 1	< 1	Mineralisation: EN 13805, mod. ICP-MS, EN 15765 mod.

Parameter (unit)	Batch number					Analytical methods
	#6	#7	#8	#9	#10	
Dioxins and PCBs						
WHO-PCDD/F-TEQ (upper bound) (pg/g of fat)	0.366	0.301	0.319	0.328	0.325	EC 2017/644, mod.
WHO-PCB + PCDD/F (upper bound) (pg/g of fat)	0.665	0.66	0.526	0.547	0.616	
ICES 6 – PCB (upper bound) (ng/g of fat)	3.33	2.64	0.37	0.38	2.86	
PAHs (μg/kg of NF)						
Benz(a)anthracene	0.5	0.6	< 0.4	< 0.4	0.5	RP-HPLC, internal
Chrysene	1.8	1.2	< 0.4	< 0.4	1.1	
Benzo(b)fluoranthene	1	0.7	< 0.4	< 0.4	0.7	
Benzo(a)pyrene	0.6	< 0.4	< 0.4	< 0.4	0.4	
PAH 4 content	3.9	2.5	< 0.4	< 0.4	2.7	
Ethanol (mg/kg of NF)	63,967	37,460	3,252	12,881	48,067	NF EN ISO 9832, mod.
Mycotoxins (μg/kg of NF)						
Ochratoxin A	< 2	< 2	< 2	< 2	< 2	UFLC, internal
Aflatoxin B1	< 2	< 2	< 2	< 2	< 2	
Aflatoxin B2	< 2	< 2	< 2	< 2	< 2	
Aflatoxin G1	< 2	< 2	< 2	< 2	< 2	
Aflatoxin G2	< 2	< 2	< 2	< 2	< 2	
Aflatoxins (B1, B2, G1, G2)	< 8	< 8	< 8	< 8	< 8	

NF: novel food; ICP-MS: inductively coupled plasma mass spectrometry; UFLC: ultra-fast liquid chromatography; WHO: World Health Organization; ICES: International Council for the Exploration of the Seas; PCDD: polychlorinated dibenzodioxins; PCDF: polychlorinated dibenzofurans; PCB: polychlorinated biphenyl; TEQ: toxicity equivalence; PAH: polycyclic aromatic hydrocarbon.

With regard to the presence of heavy metals in the NF, the maximum concentrations of lead and mercury in the NF were reported to be 0.03 mg/kg and 0.008 mg/kg, respectively. Regulation (EC) No 629/2008 for food supplements establishes upper levels of 3 mg/kg wet weight for lead and 0.1 mg/kg wet weight for mercury. Regarding cadmium, the maximum detected concentration in the NF (0.05 mg/kg) was below the limit of 3 mg/kg wet weight established by the provisions of Regulation (EC) 420/2011 for food supplements consisting exclusively or mainly of dried seaweed, products derived from seaweed or of dried bivalve molluscs. The maximum detected concentration of total arsenic in the NF has been found to be 0.10 mg/kg, below the permitted levels established by the consolidated versions of Regulation (EU) No 231/2012 for food additives and Regulation (EU) No 1881/2006 for certain contaminants in foodstuffs. The Panel notes that no specific regulatory limits are currently established in the EU regarding arsenic or tin in food supplements.

The concentrations of dioxins and PCBs in the NF are below the respective maximum levels in Regulation (EC) No 1881/2006 for dioxins and dioxin-like PCBs and non-dioxin-like PCBs in vegetable fats and oils (0.75 pg/g fat for WHO-PCDD/F-TEQ; 1.25 pg/g fat for WHO-PCDD/F-PCB-TEQ; 40 ng/g fat for ICES 6-PCB).

With regard to PAHs, levels of benzo(a)pyrene and the sum of benzo(a)pyrene, benz(a)anthracene, benzo(b)fluoranthene and chrysene were, respectively, no more than 0.6 μg/kg and 3.9 μg/kg for any of the six production batches, being below the maximal levels for benzo(a)pyrene and PAHs in oils and fats (excluding cocoa butter) intended for direct human consumption or use as an ingredient in foods, according to the provisions of Regulation (EC) No 1881/2006. The same NF batches have been screened for 250 pesticide residues and the screened compounds have been found to be below the LOD (0.01 mg/kg) of the implemented GC–MS analytical method (LFGB L 00.00–34).

The Panel noted the high variation regarding the ethanol concentration in the NF (0.33–6.4% w/w). The applicant provided data on five additional batches of the NF, more recently produced, and the ethanol concentration ranged from 0.22% to 0.58% w/w. The applicant informed EFSA that

lower and less variable ethanol concentrations were achieved upon modifying the pressure during the drying step.

Qualitative and quantitative data on the microbiological profile of the NF were provided. The average water activity of the NF has been determined as 0.446 ± 0.034 (Table 7).

Table 7: Batch-to-batch microbiological analysis of the NF

Parameter (unit)	Batch number					Analytical methods
	#6	#7	#8	#9	#10	
Total aerobic counts (30°C) (CFU/g of product)	1,800	< 100	< 100	100 (est)	< 100	XP V08-034
***Escherichia coli* (CFU/g of product)**	ND	ND	ND	ND	ND	NF ISO 16649-2
Sulfite-reducing anaerobic bacteria (46°C) (CFU/g of product)	< 10	< 10	< 10	< 10	< 10	NF V 08–061 Boîte
Coagulase-positive staphylococci (in 1 g of product)	ND	ND	ND	ND	ND	NF EN ISO 6888-3
Yeasts & moulds (CFU/g of product)	< 10	< 10	< 10	< 10	< 10	NF V08-059
***Salmonella* (in 25 g of product)**	ND	ND	ND	ND	ND	BKR 23/07–10/11
***Listeria monocytogenes* (in 25 g of product)**	ND	ND	ND	ND	ND	AES 10/03–09/00
***Vibrio* potentially enteropathogenic (in 25 g of product)**	ND	ND	ND	ND	ND	NF EN ISO 21872-1
***Vibrio cholerae* (in 25 g of product)**	ND	ND	ND	ND	ND	NF EN ISO 21872-1
***Vibrio parahaemolyticus* (in 25 g of product)**	ND	ND	ND	ND	ND	NF EN ISO 21872-1
Enterobacteriaceae (CFU/g of product)	< 10	< 10	< 10	< 10	< 10	3 M 01/06–09/97
***Bacillus cereus* (CFU/g of product)**	< 10	< 10	< 10	< 10	< 10	BKR 23/06–02/10
Water activity (25°C)	0.501	0.422	0.416	0.417	0.454	Dew point method

NF: novel food; est: estimated; CFU: colony forming unit.

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 of the NF is not complete and may raise safety concerns.

3.4.1. Stability

The applicant proposes for the NF a shelf life of 18 months, under 5°C. Stability studies have been carried out on four independently produced batches of the NF, for a period of 18 months (5 ± 3°C). The applicant clarified that relative humidity (RH) was not controlled as part of the stability studies. Peroxide value (Table 8), FX levels (Table 10), and microbiological parameters (Table 11) were selected by the applicant as markers of the NF's stability. Additionally, the levels of alpha-linolenic acid (ALA), stearidonic acid (SDA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) have been monitored (Table 9). The applicant informed of the addition of a tocopherol-rich extract to the NF (0.5% w/w), to improve its stability.

The nature of the NF renders it prone to lipid oxidation. Long-chain polyunsaturated FAs (LC-PUFAs) that comprise ca. 4–10% w/w of the NF are sensitive to temperature, light, and the presence of oxygen due to the large number of double bonds and their position within the FA chain (Albert et al., 2013). Oxidation of LC-PUFAs may have a negative impact on both quality and safety of the NF, due to the formation of compounds of hazardous potential.

Table 8: Peroxide value of the NF during storage (5°C)

Time (months)	Peroxide Value ($meqO_2/kg$)			
	#6	#7	#8	#9
0	0.2	0.3	0.1	0.1
3	0.4	2.2	0.2	0.2
6	0.3	0.3	0.5	0.5
9	0.1	0.2	0.2	0.2
12	0.1	0.1	0.2	0.1
18	0.1	0.4	0.5	0.1

$meqO_2$: milliequivalent of oxygen.

The Panel notes that the peroxide values during storage do not raise safety concern and that they are below the proposed specification limit (Table 12).

Table 9: LC PUFA levels in the NF during storage (5°C)

Time (months)	0				18			
Parameter (mg/100 g of NF)	**#6**	**#7**	**#8**	**#9**	**#6**	**#7**	**#8**	**#9**
Total n-3	6,002	6,211	6,235	5,229	5,411	5,307	5,803	4,834
ALA	82	154	114	105	130	124	96	86
EPA	5,557	5,513	5,823	4,856	5,022	4,863	5,421	4,492
DHA	164	341	179	158	148	174	185	160
SDA	56	102	59	45	41	84	48	40
Total n-6	885	1,076	1,123	1,032	801	850	934	849

LC-PUFA: long-chain polyunsaturated fatty acid; NF: novel food; ALA: alpha-linolenic acid; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; SDA: stearidonic acid.

With regard to the LC PUFA content, the Panel notes that there is an average decrease of 10% ± 3 of the total n-3 FAs, and an average reduction of 16% ± 5 of the total n-6 FAs during storage.

Table 10: Fucoxanthin levels in the NF during storage (5°C)

Time (months)	Fucoxanthin (% w/w in the NF)			
	#6	#7	#8	#9
0	2.06	2.05	2.01	2.11
3	1.89	2.04	1.92	1.90
6	1.80	2.02	1.91	1.89
9	1.71	2.06	1.91	2.03
12	2.02	2.04	1.87	1.97
18	1.77	1.85	1.76	1.91

NF: novel food.

Zhao et al. (2014) have reported that FX, the major carotenoid in the NF (1.6–2.4% w/w), is sensitive to heat and light, factors that may lead to both decrease of FX levels in the NF, and to *cis*-isomerisation of FX in the NF. The Panel notes that there are no substantial changes during storage and that the reported values in Table 10 are within the proposed specification range for FX.

The applicant provided an analysis of microbiological parameters, in four batches of the NF at t = 18 months (Table 11) (and also at intermediate time points). The Panel notes that the values do not raise safety concern and that they are below the proposed specification limits, when applicable.

Table 11: Microbiological profile of the NF during storage (5°C)

Parameter (unit)	#6	#7	#8	#9	Analytical method
Total aerobic plate at 30°C (CFU/g)	100 (est)	< 10	< 10	< 10	XP V08-034
Yeast and moulds (CFU/g)	/	< 10	< 10	< 10	NF V08-059
Enterobacteriaceae 37°C (CFU/g)	< 10	< 10	< 10	< 10	3 M 01/06–09/97
E. coli (β-Glucuronidase +)(CFU/g)	ND	ND	ND	ND	NF ISO 16649-2
Anaerobic sulfite-reducing bacteria at 46°C (CFU/g)	< 10	< 10	< 10	< 10	NF V 08–061
Coagulase-positive Staphylococci (/1 g)	ND	ND	/	/	NF EN ISO 6888-3
Pseudomonas aeruginosa (in 1 g)	/	ND	/	/	NF EN ISO 22717
Bacillus cereus presumed (CFU/g)	<10	<10	<10	<10	BKR 23/06–02/10
Salmonella (/25 g)	ND	ND	ND	ND	BKR 23/07–10/11
Listeria monocytogenes (/25 g)	ND	ND	/	ND	AES 10/03–09/00
Potentially enteropathogenic *Vibrio* (/25 g)	ND	/	/	/	NF EN ISO 21872-1
Vibrio parahaemolyticus (/25 g)	ND	/	ND	ND	NF EN ISO 21872-1
Vibrio cholerae (/25 g)	ND	/	ND	ND	NF EN ISO 21872-1

NF: novel food; est: estimated; CFU: colony forming unit; ND: not detected

The Panel notes that the applicant did not provide analytical data for secondary oxidation, using as an argument that *p*-anisidine cannot be measured due to the dark colour of the extract.

3.5. Specifications

The specifications of the NF are indicated in Table 12.

Table 12: Specifications of the novel food

Description: Ethanolic extract of *Phaeodactylum tricornutum* dried biomass		
Source: *Phaeodactylum tricornutum*		
Parameter	**Unit**	**Specification value**
Appearance	–	Dark brown-green liquid
Peroxide value	($meqO_2$/kg of fat)	< 5
Composition of the product		
Fucoxanthin	% w/w of the product	1.6–2.4
Total Fat	% w/w of the product	70.0–85.0
Protein	% w/w of the product	7.0–15.0
Ash	% w/w of the product	3.0–7.5
Moisture	% w/w of the product	≤ 2
Microbiological parameters		
Aerobic colony count at 30°C	CFU/g	< 10^4
Yeasts & Moulds	CFU/g	< 100
Salmonella	In 25 g of product	Not detected
Coagulase-positive staphylococci	In 1 g of product	Not detected
Escherichia coli	In 1 g of product	Not detected
Enterobacteriaceae	CFU/g	< 100
Residual solvents		
Ethanol	g/100 g	< 10

CFU: colony forming unit.

Regarding the specification limits for coagulase-positive staphylococci and *Escherichia coli*, the applicant proposed that the limits are 'not detected in 1 g of product'. However, the Panel notes that a more appropriate limit would be 'not detected in 10 g of product', for both the aforementioned microbiological parameters. Additionally, the Panel considered that specification limits for PHA, PPHA, BMAA, DAB, dioxins, and PCBs would be relevant for the safety of this NF. Moreover, considering the

ethanol residual levels (0.22–0.58% w/w) in the more recently produced NF batches, the Panel is of the view that the proposed specification limit for ethanol could be lower.

The Panel considers that the information provided on the specifications of the NF is not sufficient.

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

There is no history of use of the NF or its source as food. *P. tricornutum* biomass has been used as feed in aquaculture (Enzing et al., 2014).

3.7. Proposed uses and use levels and anticipated intake

3.7.1. Target population

The target population proposed by the applicant is the general adult population, excluding pregnant and breastfeeding women.

3.7.2. Proposed uses and use levels

The applicant intends to market the NF as an ingredient in food supplements at a maximum dose of 437 mg per day, corresponding to 6.24 mg/kg bw per day for adults.

3.7.3. Estimated exposure to undesirable substances

The neurotoxin DAB was quantified (0.41 mg/kg = 410 μg/kg) in one of the NF samples. In light of the results (Section 3.4 Compositional data), the applicant provided literature data on the occurrence of BMAA and DAB in other foods. Total and free DAB have been quantified in molluscs (mussels, oysters, and others), in fresh and boiled lobster, as well as in blue crab in concentrations mostly below 5 μg/g but ranging up to 15.67 μg/g (Lance et al., 2018). Additionally, the applicant provided literature data on the occurrence of DAB in mussels (*Mytillus galloprovincialis*) and oysters (*Crassostrea gigas*) for other studies (Réveillon et al., 2014; Réveillon et al., 2015). In all samples analysed, in both studies, DAB was quantified (mussels from 0.08 to 1.2 mg/kg, oysters from 0.03 to 0.60 mg/kg).

The applicant provided information on the DAB levels in seven other food supplements/food ingredients, retrieved from the work of Aparicio-Muriana et al. (2023). Among the samples tested, the concentration of DAB ranged from 'not detected' up to 2,408 μg/kg. Considering the proposed uses of the food supplements, the applicant reported that the DAB exposure would range from 0 to 5.22 μg/day. Considering the maximum dose proposed by the applicant of 437 mg of NF per day, and the highest level of DAB of 0.41 μg/g (quantified in only one out of the five analysed NF batches), the resulting exposure of an individual to DAB is 0.18 μg/day. Thus, the resulting maximum daily exposure to DAB from those samples is substantially higher than the one resulting from the consumption of the NF.

The applicant, using the literature values for BMAA and DAB attempted to estimate the minimum and maximum daily exposure of adult consumers to BMAA and DAB through the consumption of mussels, oysters, crabs, clams, and oysters, concluding that the potential exposure of the target population to these compounds would not be substantial when compared to the background diet intake.

PHA and PPHA, two degradation products of chlorophyll, are known to induce phototoxicity in humans (Tamura et al., 1979, Hwang et al. 2005), rats (Endo et al., 1982), and mice (Tamura et al. 1979).

In a 90-day rat study (Endo et al., 1982) PHA induced phototoxicity and mortality (50%) at 9.4 mg/kg bw per day, with no observed adverse effect level (NOAEL) at 4.7 mg/kg bw per day. PPHA exposure induced signs of phototoxicity and mortality (17%) at 0.78 mg/kg bw per day with NOAEL at 0.39 mg/kg bw per day (Endo et al., 1982).

Hwang et al. (2005) showed that in an outbreak of food poisoning, samples of dried laver that had been consumed were causing phototoxicity in skin patch tests in humans. The dried laver had concentrations of PHA and PPHA ranging from 89 to 90.6 mg/100 g and 546 to 562.4 mg/100 g, respectively. The amount of laver consumed that caused food poisoning was not given.

The content of PHA and PPHA reported in the NF ranged from 1.6 to 3.3 g/100 g (16,000–33,000 mg/kg) and 0.041 to 0.268 g/100 g (410–2,680 mg/kg), respectively.

Given the maximum dose of the NF of 437 mg/day as proposed by the applicant and the maximum content of PHA and PPHA in the NF, the intake of PHA is 14.4 mg/day (0.21 mg/kg bw per day[5]) and

[5] Body weight 70 kg in adults (EFSA Scientific Committee, 2012).

of PPHA 1.2 mg/day (0.017 mg/kg bw per day). The MoE to the NOAELs for PHA and PPHA derived from Endo et al. (1982) are 22 and 23, respectively.

The applicant provided estimates of the intake of PHA and PPHA derived from the consumption of other food supplements available on the international market that ranged from 4.3 to 22.7 mg/day for PHA and 15 to 1,050 mg/day for PPHA.

The Panel notes that the intake of PHA and PPHA from the NF is substantially higher (~ 38 folds) compared to the maximum 95th percentile intake in adults (i.e. 0.6 mg per day) calculated based on the concentration data in certain selected foods reported by Viera et al. (2022) and consumption data in EU dietary surveys (Annex A). The foods reported by Viera et al. (2022) were matched to broader FoodEx2 categories, to allow for an exposure estimate that includes more food items (Table 13). In case any of the reported foods would fall under the same FoodEx2 category, then the max PHA concentration was used in the exposure assessment. The Panel acknowledges that there may be other foods consumed, that could contain PHA, and were not studied by Viera et al. (2022). Nevertheless, the Panel notes that matching the studied foods to broader FoodEx2 categories, in order to perform the exposure assessment, partially mitigates this aspect.

Table 13: Pheophorbide A concentrations in selected foods - attributed FoodEx2 codes

Food (Viera et al., 2022)	FoodEx2 category	FoodEx2 code	Pheophorbide A max (mg/100 g of food) (Viera et al., 2022)
Avocado juice	Fruit juices (100% from named source)	A0BY4	0.067
Basil hummus	Hummus	A03VN	0.076
Creamed spinach	Leafy vegetables	A00KR	0.020
Green tea chocolate	Chocolate and similar	A0EQD	0.019
Guacamole	Avocados	A01LB	0.076
Kiwi juice	Fruit juices (100% from named source)	A0BY4	0.002
Pesto sauce	Pesto	A044V	0.016
Tortellini	Fresh stuffed pasta	A007T	0.010
Vegetable pasta	Dried pasta	A007L	0.086

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

The NF is a complex mixture derived from ethanolic extraction of the dried biomass of *P. tricornutum* and is standardised to 2% of FX. The NF is composed of 78% fat and 10% proteins. Since these are nutritional constituents normally present in the diet, the ADME assessment focuses on FX.

The applicant provided a specific ADME study performed in parallel to a 90-day study in Sprague–Dawley rats. In this study, rats were given the NF (containing 2.1% FX) via oral gavage at 750, 1,250, and 2,500 mg/kg day or with vehicle only (MCT-oil based on coconut oil) (Unpublished, 2018c). A satellite group of six animals (three males and three females) for each treated group was added to the study to collect data on FX and its metabolites fucoxanthinol (FXOH) and amarouciaxanthin A (Ama A). Blood was collected on day 1 and on week 13 during the treatment period at 4, 8, 12, 16, and 24 h after administration of the NF.

FX was not detected in any treated group at any time point.

FXOH, derived by hydrolysis of FX, was the most abundant metabolite. The maximum levels (T_{max}) were detected between 4 and 8 h after treatment with a mean half-life of 7 h in all treated groups. The increases in the systemic levels of FXOH were not clearly proportional to the dose increase and were less than expected. Based on the calculated AUC the exposure at week 13 was higher compared to day 1 (accumulation ratios ranged from 1.1 to 2.6). Levels of FXOH were slightly higher in females.

A second peak of FXOH was detected on week 13 after 12 h (3 animals), 16 h (1 animal), and 24 h (2 animals) post-dosing.

Ama A, derived by dehydrogenation and isomerisation of FXOH, was detected on day 1 and in week 13 in the high-dose treated animals, while in the mid- and low-dose groups, Ama A was detected only on week 13. T_{max} of Ama A was between 8 and 12 h after treatment; the levels increased more than proportionally compared to treatment doses. Higher levels were detected in males and there was an accumulation from day 1 to week 13.

The metabolism of FX is described in several *in vitro* and *in vivo* studies available in the scientific literature.

In general, dietary FX is hydrolysed to FXOH in the gastrointestinal tract by the digestive enzymes and absorbed by the intestine (Gille et al., 2018; Sugawara et al., 2002; Gammone and D'Orazio, 2015). FXOH is converted into Ama A by dehydrogenation and isomerisation in the liver (Asai et al., 2004).

Mice studies have shown that, after single dose administration, FX metabolites are accumulated in the liver, lung, kidney, heart, spleen, and adipose tissue. FXOH is detected at higher levels in the liver after single-dose administration, while after 1-week of treatment the concentration of FXOH is higher in the heart. The concentration of Ama A is always higher in the adipose tissue independently of the duration of the treatment (Hashimoto et al., 2009).

It has been reported that the FX absorption rate can be influenced by the composition of the food matrix and that FX was indicated to be poorly bioavailable when administered as vegetables (Asai et al., 2008).

The pharmacokinetics of FXOH have been evaluated in humans by Hashimoto and colleagues (Hashimoto et al., 2012) after oral administration of 'kombu' extract containing 31 mg of FX. Blood plasma was analysed by HPLC in samples collected 5 min before and 0.5, 1, 2, 4, 8, and 24 h after the treatment. A maximum FXOH concentration (C_{max}) of 44.2 nmol/L was detected with maximum concentration (T_{max}) at 4 h and a terminal half-time ($t_{½}$) of 7.0 h. Ama A and FX were not detected in the volunteers' plasma. However, a peak suspected to represent the cis-isomer of FXOH was identified.

Data indicate that the rate of metabolism for FXOH is similar among mice, rats, and humans with a T_{max} of 4 h (Hashimoto et al., 2009; CitoxLab; Hashimoto et al., 2012), while Ama A has a T_{max} of 4 h in mice and 8–12 h in rats (Hashimoto et al., 2009; CitoxLab) and was not detected in humans, suggesting that metabolism of Ama A may be different between rodents and humans.

3.9. Nutritional information

The NF is a complex mixture derived from the ethanolic extract of the microalga's *P. tricornutum* dried biomass, with a content of total FX ranging up to 2.4% (w/w). On average, the NF is composed of 78% fat and 10% crude protein. On average, 100 g of NF contain ca. 45 g of saturated FAs, and 18 g of unsaturated FAs, including 6.5 g of omega 3 and 1.1 g of omega 6 FAs. The applicant provided analytical data on the nutrient (vitamins and minerals) and amino acid profile of the NF. At the proposed use level (max 437 mg/day, Section 3.7.2), the contribution of the NF to the intake of these components is only minor.

The Panel considers that the nutrient content of the NF does not raise concerns. However, in consideration of safety concerns related to other compounds in the NF (see Discussion section), the Panel cannot conclude that the NF is not nutritionally disadvantageous.

3.10. Toxicological information

The applicant provided four toxicological studies on the NF, which were conducted in compliance with OECD principles of Good Laboratory Practice (GLP) (OECD, 1998a) and in accordance with the test guidelines No 471, 487 and 408 from the Organisation for Economic Co-operation and Development (OECD, 1997; 1998b; 2016). Additionally, the applicant provided four mechanistic studies. All studies, which were claimed proprietary by the applicant, are listed in Table 14.

Table 14: List of toxicological studies with the NF

Reference	Type of study	Test system	Dose
Study No. 45339 MMO (Unpublished, 2018a)	Bacterial reverse mutation test (GLP, OECD TG 471)	*S.* Typhimurium TA98, TA100, TA102, TA1535 and TA1537	Up to 5,000 μg/plate (absence and presence of S9 mix)
Study No. 45341 MNV (Unpublished, 2018b)	*In vitro* micronucleus test (GLP, OECD TG 487)	L5178Y $TK^{+/-}$ mouse lymphoma cells	Up to 200 μg/mL (absence and presence of S9 mix)
Study No. FSR-IPL 201001 (Unpublished, 2021a)	*In vitro* micronucleus test (GLP, OECD TG 487)	Human lymphocytes	Up to 1,000 μg/mL (absence and presence of S9 mix)
Study No. 45344 TCR (Unpublished, 2018c)	90-day repeated dose oral toxicity study (gavage) (GLP, OECD TG 408, limit test)	Sprague–Dawley rats	750, 1,250 and 2,500 mg/kg bw per day

Reference	Type of study	Test system	Dose
Study No. 46705 EPR (Unpublished, 2018d)	Interference assessment of fucoxanthinol for bilirubin dosage in plasma from rats	*In vitro* method	Rat plasma from 90-day repeated dose oral toxicity
Study No. SR-EU-274-0221 (Unpublished, 2021b)	Molecular Imaging Study of microgranuloma composition in Rat Liver and Ganglion	Histopathological samples of the 90-day study	
Study No. SR-EU-274-0221 (Unpublished, 2021c)	Molecular Imaging Study of microgranuloma composition in Rat Liver and Ganglion	Histopathological samples of the 90-day study	
Study number 48722 TSR (Unpublished, 2021d)	3-Week Mechanistic Study by the Oral Route (Gavage) in Rats	Sprague–Dawley male rats	Adrenal function in rats after ACTH challenge following daily oral administration of 2,500 mg/kg bw per day

bw: body weight; ACTH: adrenocorticotropic hormone.

3.10.1. Genotoxicity

A bacterial reverse mutation test in compliance with OECD Principles of GLP of 1997 (with the exception of the chemical analysis of dose formulation) and OECD TG 471 (OECD, 1997) was performed with the NF in *S*. Typhimurium strains TA1535, TA1537, TA98, TA100, and TA102 (Unpublished, 2018a). A preliminary experiment and two main experiments were conducted.

Based on the findings of the preliminary test the first main experiment was performed with the plate incorporation treatment at concentrations of 20.58, 61.73, 185.2, 555.6, 1,667, and 5,000 μg/plate, in the presence and absence of S9. In the absence of S9, precipitation was observed at concentrations ≥ 555.6 μg/plate and toxicity was detected in TA98 (at 5,000 μg/plate) and in TA102 (≥ 1,667 μg/plate). In the presence of S9, precipitation was present at concentrations ≥ 1,667 μg/plate, and toxicity was observed in TA98 and TA 1535 at 5,000 μg/plate.

The second main experiment was performed with the plate incorporation method at concentrations of 125, 250, 500, 1,000, and 2,000 μg/plate in the absence of S9. In the presence of S9, the treatment was performed with the pre-incubation method at the following concentrations: 250, 500, 1,000, 2,000, and 5,000 μ/plate. Precipitate was observed at concentrations ≥ 1,000 μg/plate both in the absence and in the presence of S9 and in the presence of S9 a moderate toxicity was observed in all strains at 5,000 μg/plate.

The NF did not cause a biologically relevant increase in the mean number of revertant colonies compared to the negative control (ethanol) in any strains tested, both in the absence and in the presence of metabolic activation.

The NF was also evaluated for its potential to induce micronuclei (MN) in L5178Y $TK^{+/-}$ mouse lymphoma cells in a study in compliance with the OECD Principles of GLP of 1998 (with the exception of the chemical analysis of dose formulation) and with OECD TG 487 (OECD, 2016; Unpublished, 2018b). A main experiment was performed in duplicate with concentrations selected based on a preliminary cytotoxicity test. The cells were incubated with the NF at concentrations from 12.5 to 300 μg/mL, either for 3 h in the absence and presence of metabolic activation and further incubated for 24 h in fresh medium (short-term treatment), or for 24 h without metabolic activation (long-term treatment). Precipitation was observed at 300 μg/mL and at 200 μg/mL for short-term and long-term treatment, respectively. Short-term treatment with 50 μg/mL of the NF in the presence and absence of metabolic activation induced 51% and 53% of cytotoxicity, respectively, and the MN were scored for the samples at concentrations of 12.5, 25, and 50 μg/mL.

A dose-related increase was observed after treatment for 3 h in the absence of S9. Although, the increase in MN was not statistically significant compared to the control, the number of MN detected at the highest dose was outside the historical control range.

In the presence of metabolic activation for 3 h plus recovery treatment, an increase in MN was detected at the highest dose of 50 μg/mL. The increase was not statistically significant, however, the frequency of MN cells in the vehicle and at the highest dose were out of the historical data range (5 and 7%, respectively vs 3.5% of historical control).

The long-term treatment without S9 with the highest dose of 300 μg/mL induced only 39% of cytotoxicity in the main test. The applicant selected for MN detections the doses of 100, 150, and 200 μg/mL where cytotoxicity was 8, 26, and 27%, respectively. No induction of MN was detected, however, the cytotoxicity for the highest dose selected was below the value recommended in the OECD TG 487 (OECD, 2016).

The Panel considered the *in vitro* MN test as equivocal and required the applicant to repeat the test.

The newly submitted *in vitro* MN test was performed in human lymphocytes in compliance with OECD TG 487 (OECD, 2016; Unpublished, 2021a). A preliminary cytotoxicity test was performed at the highest dose of 1,250 mg/mL.

In short-term treatment in the absence of S9, the concentrations of 500, 1,000, and 1,250 mg/mL were analysed for micronucleus formation (cytotoxicity detected at the highest concentration was 50.6%), while in the presence of S9, the concentrations analysed were 312.5, 625 and 1,000 mg/mL (cytotoxicity at the highest concentration was 57.7%).

The tested concentrations analysed for long-term treatment of 24 h in the absence of S9 were 156.25, 312.5, and 500 mg/mL (cytotoxicity at the highest concentration tested was 59.5%).

No statistically significant increase in the number of binucleated cells containing micronuclei was recorded at any condition and concentration tested.

Due to the characteristic of the complex mixture of the examined NF, the applicant was requested to perform genotoxicity studies examining fractions of *P. tricornutum* crude extract in accordance with EFSA guidelines on genotoxicity assessment of chemical mixtures (EFSA Scientific Committee, 2019). The applicant indicated that it would not be possible to separate crude extracts/fractions since MCT oil is added before the evaporation step. The Panel accepted the justification and considered the assessment of genotoxicity with the whole product acceptable.

Taking into account the test results provided and considering the nature, source, and production process of the NF, the Panel considers that there are no concerns regarding genotoxicity.

3.10.2. Subchronic toxicity

The applicant has provided a subchronic 90-day toxicity study in Sprague–Dowley rats given NF containing 2.1% of FX via gavage at 750, 1,250, and 2,500 mg/kg per day or with vehicle only (MCT-oil based on coconut oil) (Unpublished, 2018c). A satellite group of animals was added to conduct a toxicokinetics (TK) study (see ADME section). The study was performed following the OECD TG 408 (OECD, 1998b), modified in order to include an assessment of endocrine-related parameters (oestrous cycle, epididymal sperm motility and morphology and spermatozoa count) described in OECD TG 407, (OECD, 2018) and following GLP principles.

One female animal from the low-dose group was sacrificed on day 88 for poor clinical condition showing multicentric lymphoid neoplasm that was considered spontaneous and not related to treatment.

Clinical sign of ptyalism was observed in animals treated at the mid and high dose. The observation is considered related to the treatment.

No effect of the treatment was observed in the Functional Observation Battery and no ophthalmology findings were observed.

The oestrous cycle, epididymal sperm motility and morphology, and spermatozoa count were not affected by the treatment.

During the study, a non-statistically significant increase in bw (up to ~ 10%) was observed for the high-dose males compared to the control group. Statistically significant decreased bw values (up to ~ 10%) were observed in females at low and mid-dose on day 15 and mid-dose only on days 22, 29, 36, 64, 71, 78, and 85. No statistically significant differences were seen for food intake, but intake slightly increased in high-dosed males compared with the control group.

In haematology, decreases (about 20%) were observed in white blood cell (WBC) count in males that were statistically significant in low- and mid-dose groups and for lymphocytes and prothrombin time at the middle dose. A statistically significant increase (14%) in fibrinogen was observed in high-dose male rats. These changes were not clearly dose-related and therefore not further considered.

No significant differences have been observed between the control and treated groups in urinalysis parameters.

Several blood chemistry parameters were affected by the treatment.

Creatinine increased (11%) and calcium decreased (3.7%) in the high dose only in males. Despite being statistically significant, these changes of small effect size were not considered adverse.

A dose-dependent increase in alkaline phosphatase (ALP) in males was observed (12, 21, 47%, at low, mid and high dose, respectively) and was statistically significant at the high dose. A significant increase (29%) was also seen for alanine aminotransferase (ALT) in the high-dose males. These changes were considered treatment-related and adverse.

Albumin/globulin ratio and triglycerides decreased in males in a dose-dependent way and were statistically significant in the mid and highest doses and at the highest dose, respectively.

A dose-dependent increase of total bilirubin was seen in males and females, being statistically significant in the mid- (113% in male and 53% in female) and high- (148% in male and 177% in female) dose groups. The applicant suggested that the observed increase is due to the methodology used in which FXOH interferes with the measurement of total bilirubin. This has been confirmed by the applicant in a separate study (Unpublished, 2018d). The Panel agrees with this explanation.

Cholesterol increased in all male and female dose groups in a dose-dependent way (88, 109, and 134% in males and 48, 61, and 72% in females). The findings are considered as treatment-related. The values recorded in the female control group were slightly higher than those from the historical control data.

Studies conducted with FX in rodents reported similar observations regarding cholesterol concentrations (Beppu et al., 2009; Woo et al., 2010; Ha and Woo, 2013). Beppu et al. (2012) in a study conducted in diabetic/obese KK-Ay mice fed FX, observed that cholesterol increased in plasma via significant induction of sterol regulatory element binding protein 1 and 2 (SREBP 1 & 2) expression (key transcriptional factors involved in up-regulation of cholesterol biosynthesis), while at the same time, there was a significant down-regulation of low-density lipoprotein (LDL) receptor and scavenger receptor class B type 1 (SR-B1), resulting in reduced cholesterol uptake in the liver. In this study, the hepatic cholesterol content was significantly decreased, supporting the hypothesis of an increase of plasma cholesterol due to a down-regulation of serum clearance of cholesterol (i.e. decreased hepatic uptake).

Considering that no signs of cholestasis were observed at the microscopic examination, the Panel did not consider this effect as adverse.

Orange or yellow discoloration of the adipose tissue was observed in almost every dosed animal, which is most likely due to the accumulation of FX or its metabolites. Discoloration by itself is not considered adverse.

A significant dose-dependent increase in absolute heart weight was recorded for male rats at mid (27%) and high (32%) doses. The absolute and relative to body weight thyroid gland weight was significantly increased in mid-dose (18%) males and high (23%) dose females, respectively. No histopathological changes were observed in the thyroids.

Weights of adrenal glands increased dose-relatedly and were statistically significant in the mid- and high-dose groups in both sexes. The absolute adrenal weight increase was 15, 31 and 65% in males and 15, 22 and 45% in females, adrenal weight relative-to-body increased by 10, 25 and 50% in males and 21, 32 and 46% in females, while adrenal weight relative-to-brain weight increased by 14, 30 and 60% in males and 14, 24 and 45% in females, compared to control animals.

Cortical hypertrophy of adrenal glands was observed in male rats at all doses tested (5/10 at low, 6/10 at mid and 10/10 at high dose) and in female rats at the mid (6/10) and high (10/10) doses. All histological changes were classified as minimal except for one male and three females at the high dose classified as slight. Together with the increased adrenal weight seen in the mid and high-dose male and female groups, the Panel considers this effect to be of potential concern.

In histopathology, the presence of microgranulomas was observed in the liver, mesenteric lymph nodes and spleen. In the mesenteric lymph node, microgranulomas were detected at mid-dose (7/10 in males and 1/10 in females) and at high-dose (9/10 both in males and females). In the spleen, microgranulomas were present only in males at the highest dose (2/10) while in the liver, they were present in both sexes at the high dose (9/10 in males and 6/10 in females). No presence of microgranulomas was observed in the control groups.

The applicant postulated that the microgranulomas were due to engulfed test items and provided additional studies (Unpublished, 2021b,c). These studies did not provide evidence that microgranulomas were composed of NF-derived compounds.

After EFSA's request to demonstrate that the histopathological changes in the adrenal glands do not alter the adrenal function, the applicant provided a mechanistic study to evaluate the adrenal function

in rats after adrenocorticotropic hormone (ACTH) challenge performed according to Harvey et al. (2007). (Unpublished, 2021d).

In brief, two groups of nine or six Sprague–Dawley male rats were treated with 2,500 mg/kg bw per day of NF for 3 weeks, while a control group of nine rats received MCT-oil based on coconut oil. Mean corticosterone levels measured at 7 days before the treatment ranged from 122 ± 67 to 205 ± 74 ng/mL. At the end of the treatment, the control group and one treated group were challenged with 250 μg/kg bw of ACTH by the subcutaneous route 1 h after the last treatment, and corticosterone levels were measured in all groups. The control group and the treated group challenged with ACTH showed corticosterone levels of 142 ± 94 and 118 ± 74 ng/mL, respectively. The corticosterone level of the unchallenged treated group was 50 ± 29 ng/mL. The applicant concluded that treatment with the NF did not inhibit the response to ACTH in increasing the corticosterone levels. However, considering the high variability in baseline levels of cortisone in treated animals and the lack of an unchallenged control group, the Panel considers this study not sufficient to demonstrate that the NF does not alter the functionality of adrenal glands after ATCH stimuli.

Based on the histological findings in the adrenal glands observed at the low dose in male animals, the Panel concludes that 750 mg/kg bw per day was the LOAEL.

Additional studies on acute and subchronic toxicity and efficacy of FX or FX-containing food have been reported by the applicant. The Panel does not consider them relevant since they were either of short-term duration or not representative of the NF.

3.10.3. Human data

No studies or published data are available for the ethanolic extract of *P. tricornutum*. Human studies have been performed with FX derived from different sources. The Panel considers that the test material is not representative of the NF and therefore no conclusions can be drawn from this study/ these studies on the safety of the NF.

3.11. Allergenicity

The NF contains on average 10% of protein. The applicant performed the identification of proteins by LC–MS/MS in the harvested frozen biomass of the *P. tricornutum,* an intermediate product in the NF production. The results were compared to proteins from *P. tricornutum* database, common allergenic proteins, and common mass spectrometry contaminants mined from UniProt KB. 10,513 peptides, corresponding to 2246 proteins identified in the frozen biomass, and one peptide was found to be in common with the allergens database.

The Panel notes that the intermediate product contains a recognised allergen (triosephosphate isomerase) whose structure is shared with other taxa, among which *Crangon crangon* (Cra c 8), a shrimp species.

Based on available data and given the protein content of the NF (10 g/100 g), allergic reactions may occur upon consumption of the NF, especially in shrimp-allergic individuals.

4. Discussion

The NF, which is the subject of the application, is an ethanolic extract of the dried biomass of the microalga *P. tricornutum* diluted in an MCT oil carrier. The main component of the NF is fat (78% on average), followed by crude protein (10% on average).

In 2019, the EFSA BIOHAZ Panel assessed the *P. tricornutum* and did not grant the QPS status due to the lack of a safe history of use in the food chain and its potential for production of bioactive compounds with toxic effects. The Panel considers that based on the information provided, the microalga does not survive the manufacturing process.

The applicant intends to market the NF as a food supplement at the use level of 437 mg/day. The target population proposed by the applicant is the adult population, excluding pregnant and lactating women. There is no history of use of the NF or of its source, i.e. *P. tricornutum*.

The cyanotoxin DAB was quantified in one of the NF batches. Considering the body of evidence provided by the applicant regarding the absence of cyanobacteria in the production process, together with the quantification of DAB in only one of the analysed NF batches, it is unclear what the source of DAB is. Moreover, the Panel notes that the FFA content varied substantially among batches (13.12 ± 4.77%). In light of the above, the Panel is of the view that a consistent and safe production process has not been demonstrated.

Additionally, the Panel notes that there are uncertainties regarding the analytical methods developed and implemented for the quantification of the chlorophyll derivatives (e.g. PPHA); thus, it cannot be concluded that compounds with phototoxic potential have been sufficiently characterised. The Panel considers that the information provided on the composition of the NF is not complete and may raise safety concerns.

The Panel considers that there is no concern with respect to the genotoxicity of the NF. The applicant submitted a subchronic 90-day toxicity study with the NF as test material. In the 90-day study, a number of adverse effects were observed, of which an increase in adrenal weight together with histopathological adrenal cortical hypertrophy were the most sensitive endpoints. These effects were seen already at the lowest dose tested (750 mg/kg bw per day), which was identified by the Panel as the LOAEL.

The Panel notes the potential phototoxicity of PHA and PPHA present in the NF and the low MoE calculated for these substances at the proposed use levels, as indicated in Section 3.7.3.

The Panel also notes that phototoxicity was not addressed in the 90-day study conducted with the NF. Although it is high uncertainty in the analytical determination of these substances in the NF (in particular for PPHA) and limitations in the publicly available data on the phototoxicity of these substances, the Panel considers phototoxicity of concern.

The Panel considers that the safety of the NF has not been established.

5. Conclusions

The Panel concludes that the safety of the NF, an ethanolic extract of the dried biomass of the microalga *P. tricornutum*, has not been established.

6. Recommendation

The Panel recommends investigating the occurrence of phototoxicity after consumption of food supplements available on the EU market, that contain relatively high concentrations of chlorophyll degradation products.

7. Steps taken by EFSA

1) On 10 May 2019 EFSA received a letter from the European Commission with the request for a scientific opinion on the safety of the extract of *Phaeodactylum tricornutum* as a novel food. Ref. Ares (2019)3100793 - 10/05/2019.
2) On 10 May 2019, a valid application on the extract of *Phaeodactylum tricornutum* as a novel food, which was submitted by Microphyt, was made available to EFSA by the European Commission through the Commission e-submission portal (NF 2018/0626) and the scientific evaluation procedure was initiated.
3) On 22 November 2019, 10 June 2021, 14 October 2021, 17 February 2022, 13 May 2023, and 8 November 2022, EFSA requested the applicant to provide additional information to accompany the application and the scientific evaluation was suspended.
4) On 31 May 2021, 30 September 2021, 9 February 2022, 15 April 2022, 7 November 2022, and 19 May 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 24 May 2023, the NDA Panel, having evaluated the data, adopted a scientific opinion on the safety of the ethanolic extract of the dried biomass of the microalga *Phaeodactylum tricornutum* as a novel food pursuant to Regulation (EU) 2015/2283 as a NF pursuant to Regulation (EU) 2015/2283.

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Abbreviations

A/G	albumin/globulin ratio
ACTH	adreno corticotropic hormone
ADME	absorption, distribution, metabolism and excretion
ALA	alpha-linolenic acid
ALP	alkaline phosphatase
ALT	alanine amino transferase
Ama A	amarouciaxanthin A
ANSES	Administración NAcional de la Seguridad Social (National Social Security Administration, Spain)
ASU	Amtliche Sammlung von Untersuchungsverfahren (Official Collection of Methods of Analysis)
AUC	area under the curve
AZA	azaspiracid
BIOHAZ	EFSA Panel on Biological Hazards
BMAA	β-N-methylamino-L-alanine
bw	body weight
CCAP	Culture Collection of Algae and Protozoa
CFU	colony forming unit
CHOL	cholesterol
Cmax	maximum concentration
Cra c 8	Crangon crangon
CREAT	creatinine
DAB	2,4-diaminobutyric acid
DHA	docosahexaenoic acid
EPA	eicosapentaenoic acid
EN	Europaische Norm (European Standard)
est	estimated
FA	fatty acid
FFA	free fatty acid
FIP	fibrogen
FX	fucoxanthin
FXOH	fucoxanthinol
GC-FID	gas chromatography-flame ionization detection
GC–MS	Gas chromatography–Mass spectroscopy
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
GYM	gymnodimine
HACCP	Hazard Analysis Critical Control Points
HILIC-MS/MS	Hydrophilic interaction chromatography–Tandem Mass Spectroscopy
HPLC-DAD	High-performance liquid chromatography–Diode-array detection
HPLC-ELSD	High-performance liquid chromatography–evaporative light scattering detection
HPLC–MS	high-performance liquid chromatography–mass spectrometry

HPLC-MS/MS	High-performance liquid chromatography–Tandem mass spectroscopy
HPLC-UV	High-performance liquid chromatography–ultraviolet detection
ICES	International Council for the Exploration of the Seas
ICES 6-PCB	six indicator polychlorinated biphenyls
ICP-MS	inductively coupled plasma mass spectrometry
ISO	international organization for standardization
L	lymph
LC-MS/MS	Liquid chromatography–Tandem Mass Spectrometry
LC-PUFAs	long-chain polyunsaturated fatty acids
LDL	low-density lipoprotein
LOAEL	lowest-observed-adverse-effect-level
LOD	limit of detection
LOQ	limit of quantification
MCT	medium-chain triglyceride
MN	micronuclei
MoE	margin of exposure
ND	not detected
NDA	EFSA Panel on Nutrition, Novel Foods and Food Allergens
NF	novel Food
NOAEL	no observed adverse effect level
OA	okadaic acid
OECD	Organisation for Economic Co-operation and Development
PAHs	polycyclic aromatic hydrocarbons
PCBs	polychlorinated biphenyls
PCDD	polychlorinated dibenzodioxins
PCDF	polychlorinated dibenzofurans
PHA	pheophorbide A
PPHA	pyropheophorbide A
PT	prothrombin time
PTX2	pectenotoxin 2
QC	quality control
QPS	qualified presumption of safety
RH	relative humidity
RP-HPLC	reverse-phase high-performance liquid chromatography
rRNA	ribosomal ribonucleic acid
SDA	stearidonic acid
SPX1	13-desmethyl spirolide C
SR-B1	scavenger receptor class B type 1
SREBP 1 & 2	sterol regulatory element binding protein 1 and 2
t1/2	terminal half-time
TEQ	toxicity equivalence
TG	Test Guidelines
TK	toxicokinetics
Tmax	time required to reach the maximum concentration
TOT BIL	total bilirubin
TRIG	triglycerides
UFLC	ultra-fast liquid chromatography
w/w	weight per weight
WHO	World Health Organization
WHO-PCDD/F-PCB-TEQ	total dioxin equivalency
WHO-PCDD/F-TEQ	the sum of the toxic equivalencies of the 17 most toxicologically significant dioxins and furans
YTX	yessotoxins

Appendix A – Batch-to-batch determination of the fatty acids profile of the NF

Fatty acids (mg/100 g of product)	Batch number				
	#6	#7	#8	#9	#10
Total saturated fatty acids	49,962	50,732	44,739	53,558	43,608
Caprylic acid	26,474	26,264	22,649	28,562	21,883
Capric acid	20,436	21,207	18,126	21,545	17,365
Palmitic acid	1,885	2,114	2,556	2,258	2,793
Myristic acid	717	779	840	698	986
Lignoceric acid	150	ND	274	228	286
Stearic acid	58	164	122	107	129
Lauric acid	122	139	110	120	103
Isomyristic acid	73	ND	ND	ND	ND
Heptadecenoic acid	45	98	61	40	61
Pentadecanoic acid	46	47	63	41	63
Behenic acid	ND	ND	ND	ND	ND
Arachidic acid	ND	19	ND	ND	ND
Total unsaturated fatty acids	15,797	16,321	16,921	14,714	18,474
Total monounsaturated fatty acids	5,234	5,085	5,479	5,043	6,275
Hexadecenoic acid (other isomers)	4,768	4,187	4,755	4,372	5,412
Oleic acid and its isomers	422	755	662	631	802
Gadoleic acid and its isomers	ND	45	ND	ND	ND
Nervonic acid	ND	ND	ND	ND	ND
Total polyunsaturated fatty acids	10,563	11,236	11,442	9,671	12,199
Hexadecatrienoic acid	1,848	2,266	2,454	2,053	2,255
Mead acid	ND	235	267	279	309
Hexadecatetraenoic acid	316	212	168	105	234
Octadecatrienoic acid (other isomers)	126	71	72	59	90
Total omega 3 fatty acids	6,002	6,211	6,235	5,229	6,704
Eicosapentaenoic acid (EPA)	5,557	5,513	5,823	4,856	6,138
Docosahexaenoic acid (DHA)	164	341	179	158	197
Alpha-linolenic acid (ALA)	82	154	114	105	174
Stearidonic acid (SDA)	56	102	59	45	77
Total omega 6 fatty acids	885	1,076	1,123	1,032	1,196
Hexadecadienoic acid	1,348	1,139	1,123	914	1,381
Linoleic acid	444	575	603	534	658
Arachidonic acid	397	411	418	406	436
Eicosatetraenoic acid	143	101	59	64	118
Gamma- linolenic acid	44	58	66	59	60
Eicosadienoic acid	ND	32	36	33	42
Total Conjugated Linoleic Acid (isomers)	38	26	ND	ND	30

Annex A – Dietary exposure estimates to Pheophorbide A from selected foods for each population group from each EU dietary survey

Information provided in this Annex is shown in an Excel file (downloadable at https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2023.8072#support-information-section).