

Safety Assessment of eicosapentaenoic acid (EPA)-rich oil from *Phaeodactylum tricornutum*

Name of Applicant: Simris Alg AB, Hammenhög, Sweden

Contact person(s): Rhodri Evans, Exponent International Ltd.

Novel Food Classification: 1.2 (d)

Introduction

An application for the authorisation of EPA-rich oil derived from the microalgae *Phaeodactylum tricornutum* was submitted to the Food Safety Authority of Ireland (FSAI) by Simris Alg AB of Sweden in accordance with *Article 4* of the novel food Regulation (EC) No. 258/97. The application was accepted by the FSAI on 20th December, 2016.

Omega-3 long chain polyunsaturated fatty acids (LC-PUFA's), particularly EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) are considered essential constituents in human nutrition, with some evidence for a role in the growth and development of infants and the maintenance of cardiovascular health in adults.

Fish and fish oils are a natural source of polyunsaturated fatty acids such as DHA and EPA. However, marine microalgae are the primary producers of PUFA's in the aquatic food chain and are now being exploited as a viable alternative source of PUFAs to fish oils. A number of marine algae are already authorised to produce PUFAs as novel foods in the EU (e.g. *Schizochytrium* sp., and *Ulkenia* spp.). The EPA-rich oil in this application is produced by the freshwater and marine microalgae *P. tricornutum*. The oil is derived by supercritical CO₂ extraction of freeze-dried microalgae and is intended for use as an ingredient in food supplements.

The novel ingredient differs from similar existing microalgal products in that the predominant PUFA is EPA (27% EPA, with only 0.8% DHA). The recommended daily dose of the novel ingredient will be 2g/day when consumed as a supplement in capsule form, giving a combined daily intake of 250mg of EPA and DHA.

The novel ingredient is classed by the applicant as falling under *Article 1.2(d)* of the novel food Regulation (EC) No 258/97: "foods or food ingredients consisting of or isolated from microorganisms, fungi or algae". In order to assess wholesomeness, the application dossier

was prepared pursuant to Class 2 of Commission Recommendation 97/618/EC; “Complex NF from non-GM source”, and subclass (2); “the source of the NF has no history of food use in the Community”.

I. Specification of the novel food

The novel food ingredient is EPA-rich oil (27%) derived from the microalgae *Phaeodactylum tricornutum*.

Parameter	Analytical results
Acid value (mg KOH/g)	120 - 143
Moisture and Volatiles (g/100g)	0.14 – 0.25
Peroxide value (meqO ₂ /kg Fat)	7 - 14
Total protein (g/100g)	0.13 – 0.31
Astaxanthin (mg/kg)	<100
Beta-carotene (mg/kg)	1,944 – 2,837
Total tocopherol (mg/kg)	474 – 2,586
Unsataponifiable matter (%)	13.4 – 15.4

The fatty acid profiles of 5 batches of Simris EPA oil demonstrate a consistent product with the EPA levels (21 - 27%) far exceeding those of DHA (0.5 to 0.8%) and with the monounsaturated palmitoleic acid being the most abundant fatty acid (29 - 32%). The total protein content of the EPA-rich oil ranges from 0.13 to 0.31 g/100g.

II. Effect of the production process applied to the novel food

The novel EPA-rich algal oil is produced in Sweden using a non-genetically modified microalgae (*Phaeodactylum tricornutum*). The production site is certified under an adaption of ISO 22000 for the production of food supplements in Sweden and the water used is subject to microbiological and chemical analysis. The overall manufacturing process of the novel ingredient is similar to those of existing algal and vegetable oils already on the EU market. Algae are grown in closed-system photo-bioreactors with continuous agitation. The biomass is harvested by gentle centrifugation and then ground in a bead mill to lyse the cells. Algal residues are freeze-dried and the oil derived by super-critical CO₂ extraction. The final product sold for consumption in food supplements will contain the novel ingredient blended with DHA-rich oil, flaxseed oil and antioxidants. Analysis of 5 batches of the novel ingredient demonstrates the reproducibility of the production process.

Simris EPA-rich oil is produced in line with good manufacturing practices (GMP) and has a Hazard Analysis and Critical Control Points (HACCP) system in place. The primary degradation pathway of marine and algal oils is auto-oxidation of the unsaturated fatty acids, which has a detrimental impact on organoleptic properties. The novel ingredient is blended with DHA-rich oil, flaxseed oil and antioxidants and is stable for 12 months when stored in the dark at temperatures of 15-25°C. There are three on-going stability studies including (i) long term ambient conditions (temperature ~25°C) and relative humidity (~60%), (ii) accelerated conditions (temperature ~30°C) and relative humidity (~65%) and (iii) accelerated conditions (temperature ~40°C) and relative humidity (~75%). Preliminary results have been provided for a limited set of stability studies. The initial '0 month' data is generally in line with voluntary international industry standards for PUFA nutritional supplement. However, the increase in p-Anisidine and TOTOX values evident after a few months in the accelerated studies may indicate a potential problem with the product's stability.

III. History of the organism used for the novel food

The majority of algal oils currently authorised for the EU market are produced from *Schizochytrium sp.*, or *Ulkenia sp.* DHA-rich oil from *Cryptothecodinium cohnii* was added to infant formula prior to 1997 and so does not fall within the scope of novel food. The novel EPA-rich algal oil is produced from the microalgae *P. tricornutum* which belongs to the same phylum and kingdom as *Schizochytrium sp.* but has not previously been used as a source of algal oil for food use in the EU. The Joint Research Centre (JRC) of the EU Commission has evaluated the possibility of using a range of microalgae for food production and concluded that *P. tricornutum* is non-toxic and lacks the capability to produce toxins. *P. tricornutum* is also extensively used in aquaculture as feed for fish and other seafood intended for human consumption.

IV. Anticipated intake/extent of use of the novel food

The applicant proposes to market Simris EPA-rich oil for use in food supplements. It will be blended with flaxseed oil and antioxidants as well as DHA-rich oil from *Schizochytrium sp.* which has previously been authorised as a novel food in the EU. The EPA-rich oil will be used in a blend for the general population (Simris® Algae omega-3) and a second with added astaxanthin for athletes. Each capsule will contain ≥ 25 mg EPA and 100mg DHA with an additional 2mg of astaxanthin (from the astaxanthin-rich algae *Haematococcus pluvialis*) per

capsule in the supplement for athletes. Approximate blends of fatty acids are provided, with the actual ratios depending on the blend composition of the initial EPA-rich oil; the DHA rich oil and flaxseed oil being used to standardise the composition.

Supplement	Simris EPA oil (<i>P.tricornutum</i>)	EHA oil (<i>Schizochytrium sp.</i>)	Flaxseed oil (standardisation)	Astaxanthin- rich oil (<i>H. pluvialis</i>)
Simris® Algae Omega-3	12%	30%	58%	n/a
Simris ® Algae omega-3 for athletes	12%	30%	56%	2%

The recommended daily dose of the novel ingredient is 2g/day, yielding 250mg/day of EPA and DHA which is in line with EFSA recommendations for an ‘adequate intake’ for EPA and DHA. The omega-3 fatty acid profile for a daily serving of the proposed blended food supplements is provided.

Nutrient	Simris ® Algae Omega-3 mg/2 g daily serve	Simris ® Algae Omega-3 for athletes mg/2g daily serve
Total n-3 fatty acids	978	928.8
Alpha linolenic acid (C18:3)	646	605.6
Stearidonic acid (C18:4n3)	2.6	3.4
Eicosatetraenoic acid (C20:4:n3)	5.4	4.46
EPA (C20:5n3)	72.8	63
DHA (C22:6n3)	248	249.2
DPA (C22:5n3)	2.8	3.2

IX. Information from previous human exposure to the novel food or its source

The long chain n-3 fatty acids DHA and EPA are important components of a regular diet, with intakes higher in people who consume fish and fish oil supplements. Adequate intakes established by EFSA are 250mg/day for EPA and DHA, however, estimates in Europe at the highest percentiles of intake are reported by the applicant to range from <1,200mg/day from food only to <1,300mg from food and supplement sources (EFSA 20120). Intakes of <2.7g/day in high fish consumers have been reported and up to 1.7g/day for children consuming food supplements. Algal oil has a history of use in the EU as an ingredient in

foods for the general population, in PARNUTS (particularly in infant formulae), and in food supplements products. Oil from *Schizochytrium sp.* is widely available in the EU but there is no history of exposure to EPA-rich oils extracted from *P. tricornerutum*. The Joint Research Centre (JRC) of the European Union evaluated the potential for microalgae, such as *P. tricornerutum* in food production. The conclusion of the JRC was that *P. tricornerutum* is non-toxic and lacks the capability to produce toxins. This microalgae is used extensively in aquaculture as feed for fish intended for human consumption. EPA and DHA are important components of the diet, with intakes higher in people who consume fish and fish oil-containing food supplements. Adequate intakes established by EFSA are 250mg/day for EPA and DHA.

X. Nutritional information on the novel food

EPA is listed as the primary polyunsaturated fatty acid in this novel algal oil. The nutritional value of EPA and DHA is well-established, with several EFSA reviews and the establishment of dietary guidance and dietary reference values as well as consideration of nutrition and health claims relating to normal brain function, vision and normal function of the heart. The fatty acid profile of 5 batches of the novel ingredient has been provided by the applicant. In total, the free fatty acid content ranges from 57.6-71.35g/100g. Nitrogen levels were determined in the 5 batches of the novel ingredient with observed levels of nitrogen ranging from 0.02 to 0.05g/100g which converts to a range of 0.13 to 0.31g/100g protein equivalents.

Overall, there would appear to be little concern regarding the impact on the nutritional status of individuals who might consume the novel EPA-rich oil as it will simply substitute for other sources of the same nutrient.

XI. Microbiological information on the novel food

Microbiological analysis of 5 batches has been reported by the applicant. Analysis included *Clostridium perfringens*, Coagulase positive staphylococcus, coliforms, *Escherichia coli*, moulds, *Salmonella* and yeast and no concerns were raised.

XII. Toxicological information on the novel food

The applicant has outlined a number of previous safety evaluations of similar algal oils, including those produced from *Schizochytrium sp.* and *Ulkenia sp.* The JRC (Joint Research Centre) of the European Union has concluded that the use of a range of different microalgae, including *P. tricornerutum* for food production is not of toxicological concern. EFSA has

previously assessed the safety of both EPA and DHA and more recently a positive scientific opinion on an extension of use for DHA and EPA-rich algal oil from *Schizochtrium sp* as a novel food in food supplements up to a maximum DHA and EPA content of 3g per daily dose for the adult population, excluding pregnant and lactating women.

Toxicological Studies

The applicant has not undertaken specific toxicological studies of the novel EPA-rich algal oil but provides toxicological evaluation based on published scientific literature.

Human Safety Data

Phaedactylum tricornutum is a naturally occurring algae found throughout marine and freshwater environments. A number of *in vivo* studies in the publically available literature have been reviewed and the applicant has concluded that there is no indication that *P. tricornutum* has any significant toxicological potential. There is evidence to suggest that virtually all of the components present in algal oil are already present already to a significant degree in the human food chain.

Heavy metals such as lead, cadmium, mercury, arsenic and copper are either undetectable or not present to any appreciable amounts in the novel ingredient, while the presence of low levels of iron is not a cause for concern. The levels of PAH, dioxins, dioxin-like PCBs and non-dioxin-like PCBs were below the EU maximum limits for marine oils intended for human consumption. Pesticide residues were not detected.

As a group, microalgae have been extensively tested for their toxicological properties and the applicant concludes that microalgae that produce the same fatty acid components (DHA and EPA have previously been accepted for use in foods in the EU and USA. *P. tricornutum* is not known to produce any known toxins and analysis of the novel ingredient did not detect domoic. Though some species of algae produce toxins, they tend to be members of the dinoflagellate and blue-green algal species which are in a different kingdom to that of *P. tricornutum*.

Conclusions

DHA and EPA derived from algal sources are already available in supplements and other food categories on the EU market. This application relates to EPA-rich oil derived from the microalgae *P. tricornutum* which has not been used as a source of omega-3 oils to date in the EU. The novel ingredient will be blended with already authorised DHA-rich oil and flaxseed

oil and so will be an alternative to existing similar products on the EU market. The recommended daily dose of the oil when taken as directed would result in 2g/day, giving a combined daily intake of DHA and EPA of 250 mg, which is within EFSA guidance levels. In general, the projected exposure levels, the toxicology data outlined and presuming adherence to the described specification, there is no identifiable risk associated with the intended use of the applicants' EPA-rich algal oil. The conclusion of ongoing stability studies will be useful to further clarify the stability of the product.

In the blend, the novel ingredient will be declared as 'oil from microalgae *Phaedactylum tricornutum*' and so comply with Regulation EU 1169/2011. Labelling and other requirements associated with the marketing of food supplements within the EU will automatically apply.

Recommendation

The Food Safety Authority of Ireland has not identified any significant safety concerns associated with the consumption of EPA-rich oil produced by Simris Alg AB of Sweden from the microalgae *Phaeodactylum tricornutum* at the proposed use levels in food supplements. Therefore the FSAI is of the opinion that it meets the criteria for novel food set out in *Article 3.1.* of the novel food Regulation (EC) No 258/97.