



## Report of the Scientific Committee of the Spanish Agency for Food Safety and Nutrition on a request for initial assessment for marketing of the marine microalgae *Tetraselmis chuii* under Regulation (EC) No 258/97 on novel foods and novel food ingredients

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### Abstract

The company Fitoplancton Marino S.L. requested authorization to market the microalgae *Tetraselmis chuii* in the European Union as a seafood flavouring agent. This foodstuff has no history of use in any significant quantities in the European Union prior to 1997 and therefore comes within the scope of Regulation (EC) No 258/1997 on novel foods and novel food ingredients.

The Scientific Committee of the Spanish Agency for Food safety and Nutrition (AESAN) takes the view that, according to the information provided, there is no indication that consumption of the species of marine microalgae *Tetraselmis chuii* as a condiment, under the conditions proposed by the applicant, can produce adverse effects on health. The Committee concludes that the novel foodstuff presented for assessment meets the criteria for acceptance laid down by Regulation (EC) No 258/1997 on novel foods and novel food ingredients.

### Key words

Microalgae, novel foods, *Tetraselmis chuii*.

## 1. Introduction

The company Fitoplancton Marino S.L. requested authorisation to market the microalgae *Tetraselmis chuii* in the European Union as a seafood flavouring agent.

Fitoplancton Marino S.L. wishes to market in the European Union dried *Tetraselmis chuii* in the form of a powder incorporated in salt and as an ingredient for sauces to flavour various prepared dishes.

According to the applicant, the microalgae *Tetraselmis chuii* has no history of use in the European Union prior to 1997 and therefore comes within the scope of Regulation (EC) No 258/97 on novel foods and novel food ingredients. The product is classified in Article 1(2)(d) of the said Regulation as: "foods and food ingredients consisting of or isolated from microorganisms, fungi or algae" (EU, 1997a).

The applicant declares that the novel food belongs to Class 2 "Complex novel foods from non-genetically modified sources", Subclass 2 "the source of the novel food has no history of use as a food in the Community" pursuant to Commission Recommendation 97/618/EC of 29 July 1997. As a result the report presented by the applicant follows the guidelines laid down for category 2, including sections I, II, III, IX, XI, XII and XIII in Table II of the said Recommendation (EU, 1997b).

Some microalgae, such as *Chlorella* sp., are consumed in Europe as food supplements mainly because of the proteins, essential amino acids, minerals and trace elements they contain. The applicant states that there are many similarities between *Chlorella* and the microalgae *Tetraselmis chuii* which it wishes to market in dried form.

The applicant states that the company Fitoplancton Marino S.L. has been producing the microalgae *Tetraselmis chuii* to feed the larvae of fish, crustaceans and molluscs for over eight years and, given its organoleptic characteristics, composition and lack of toxicity, it is ideal for human consumption as an ingredient in a large variety of dishes.

### Comments

The Scientific Committee agrees with the applicant's classification of the product and AESAN has verified that there is no history of consumption in the European Union prior to 1997.

## 2. Identification of the product as a novel food

### I. Specifications of the novel food

*Tetraselmis chuii* is a single-cell mobile marine microalgae between 10 and 15 µm in size, with an ellipsoidal form produced by longitudinal fission.

The taxonomic classification of microalgae *Tetraselmis chuii* Butcher (1959) is as follows:

Kingdom: *Plantae*

Phylum: *Chlorophyta*

Class: *Prasinophyceae*

Order: *Chloroderidrales*

Family: *Chlorodendraceae*

Genus: *Tetraselmis*

*Tetraselmis chuii* was isolated for the first time in the fifties on the coast of Great Britain (Butcher, 1959) and subsequently in various parts of the world, including the Bay of Cadiz. It is identified by number 8/6 in the Culture Collection of Algae and Protozoa (CCAP) of the United Kingdom.

As the applicant certifies, the strain to be marketed comes from the collection of cultures of marine microorganisms at the Institute of Marine Science in Andalusia, belonging to the Spanish National Research Council (ICMAN-CSIC), where it has been kept as a culture since it was acquired from the Culture Collection of Algae and Protozoa (CCAP).

The applicant has presented studies identifying the strain used, analyses of the composition (physico-chemical composition, aminoacids, contents of minerals and fatty acids) and an analysis of contaminants (heavy metals, pesticides and microbiological contaminants) of three batches of dried *Tetraselmis chuii* produced industrially by the company Fitoplancton Marino S.L. The applicant company is registered in the Spanish General Health Registry for Food Companies in the condiments and spices sector and has submitted a copy of certification of authorisation to perform analytical activities from the various laboratories which carried out the analyses and their accreditation in compliance with the standard UNE/EN ISO/IEC 17025. It states that microalgae are not included in the accreditation scope because they are not a common matrix for analysis but the laboratories are accredited for conducting various analyses of food products.

### Identification studies

The strain was identified by molecular techniques using two molecular markers: the nuclear marker rDNA 18S, widely used in identification studies on species of microalgae and, in addition, the plastid marker rbcL. The results of both markers were compared with those obtained in the same way from the strain of *Tetraselmis chuii* belonging to the ICMAN-CSIC collection of microalgae.

The methodology comprised PCR amplification and subsequent sequencing of the fragments obtained from five primers (Euk1A, Euk516r, EuSSUF-1, EuSSUR-1 and Nsp4) belonging to the nuclear marker rDNA 18S and to degenerated primers (Tetra\_rbcL\_F and Tetra\_rbcL\_R) belonging to the plastid marker rbcL (Table 1).

Primer	Sequence	Reference	Marker
Euk1A	CTGGTTGATCCTGCCAG	Díez et al., 2001	rDNA 18S
Euk516r	ACCAGACTGCCCTCC	Díez et al., 2011	rDNA 18S
EuSSUF-1	AACCTGGTTGATYCTGCCAG	Sakata et al., 2000	rDNA 18S
EuSSUR-1	TGATCCTTCYGCAGGTTACCTAC	Sakata et al., 2000	rDNA 18S
Nsp4	ACTAAGAACGGCCATGCACCACCAC	IFAPA*	rDNA 18S
Tetra_rbcL_F	GKACTTGACAACACTGTATGGACKGATGGT	IFAPA*	rbcL
Tetra_rbcL_R	GRTCTTTTTCWACRTAAGCATCACGCATTA	IFAPA*	rbcL

\*Instituto de Investigación y Formación Agraria y Pesquera (Institute for Research and Training in Agriculture and Fisheries).

The sequence fragments of the marker rDNA 18S were 100 % identical with the reference sequence for the strain from the ICMAN/CSIC collection and identical or 99.9 % identical values were obtained when they were compared with other sequences for the same species deposited in the database of the National Center for Biotechnology Information (NCBI).

In order to identify the strain with greater certainty, the sequence fragments were compared with those for other species of the same genus deposited in the database of the NCBI, which showed that the identity percentages were significantly lower.

The rbcL marker was 100 % identical with the reference sequence from the ICMAN-CSIC strain. When a comparison was made with other species of the same genus, the identity values were between 98.7 and 84.6 %.

### Analysis of composition

The microalgae *Tetraselmis chuii* has a high content of proteins, carbohydrates and minerals (Table 2). The proteins contain glutamic acid, aspartic acid and leucine as the most abundant amino acids, and all the essential amino acids. No data was provided on asparagine, glutamine, proline and cysteine. Calcium is the most abundant element in the dried product within the group of minerals, and chlorates and sodium are also abundant. Fats represent 6.7 % of the product and approximately 50 % of the fatty acids are polyunsaturated, the most abundant of which are linoleic acids.

**Table 2.** Results of the analysis of composition of three lots of dried *Tetraselmis chuii* produced industrially by Fitoplancton Marino S. L. (average of the three batches  $\pm$  standard deviation)

Analyses		Results
Humidity (%)		6.3 $\pm$ 0.02
Proteins (%)		37.6 $\pm$ 0.40
Ashess (%)		15.5 $\pm$ 0.05
Carbohydrates (%)		31.6 $\pm$ 0.38
Dietary fibre (%)		2.3 $\pm$ 0.00
Fat (%)		6.7 $\pm$ 0.25
Kcal/100 g		337 $\pm$ 1.35
Kjules/100 g		1 408 $\pm$ 5.66
Amino acids (% of proteins)	Valine	2.27 $\pm$ 0.12
	Tryptophan	0.61 $\pm$ 0.01
	Threonine	1.81 $\pm$ 0.13
	Tyrosina	1.38 $\pm$ 0.15
	Serina	1.63 $\pm$ 0.09
	Methionine	0.87 $\pm$ 0.12
	Lysine	2.03 $\pm$ 0.15
	Leucine	3.08 $\pm$ 0.09
	Isoleucine	1.57 $\pm$ 0.11
	Histidine	0.65 $\pm$ 0.13
	Glycine	2.25 $\pm$ 0.14
	Phenylalanine	1.95 $\pm$ 0.07
	Arginine	2.66 $\pm$ 0.09
	Alanine	2.79 $\pm$ 0.17
	Glutamic acid	4.67 $\pm$ 0.12
Aspartic acid	3.71 $\pm$ 0.25	
Minerals (mg/g)	Calcium	33.80 $\pm$ 0.26
	Magnesium	5.06 $\pm$ 0.09
	Iron	2.01 $\pm$ 0.04
	Phosphorus	6.27 $\pm$ 1.87
	Sodium	14.33 $\pm$ 4.16
	Potassium	10.40 $\pm$ 0.56
	Chlorides	17.77 $\pm$ 0.25
	Copper	0.006 $\pm$ 0.00
Iodine (mg/kg)	5.03 $\pm$ 5.78*	

**Table 2.** Results of the analysis of composition of three lots of dried *Tetraselmis chuii* produced industrially by Fitoplancton Marino S.L. (average of the three batches  $\pm$  standard deviation)

	Analyses	Results
Fatty acids (% of fat)	Saturated	30.27 $\pm$ 0.50
	Monounsaturated	22.97 $\pm$ 0.90
	Polyunsaturated	46.77 $\pm$ 1.36

**Note:** Four batches were used for analysis of the iodine concentration. The variability between the batches was attributed to seasonal changes: cultures in summer provided lower results (0.45 and 0.47 mg/kg) than the two batches from winter cultures (6.7 and 12.5 mg/kg).

### Analysis of contaminants

No contaminants were detected in any of the three batches analysed (Table 3).

Regulation (EC) No 420/2011 lays down maximum admissible limits for cadmium and lead of 0.05\* and 0.1 mg/kg fresh weight respectively in vegetables\* (EU, 2011). The criteria for the maximum content of arsenic, mercury and tin in dry material established in France for algae are 3, 0.1 and 5 mg/kg respectively (CEVA, 2012). The results of the analysis provided by the applicant show the concentrations of heavy metals to be below these maximum limits.

No pathogenic microorganisms were detected during the microbiological analysis. The content of mesophilic aerobic bacteria will be discussed in Section XII.

**Table 3.** Results of the analysis of contaminants of dried *Tetraselmis chuii* produced industrially by Fitoplancton Marino S.L. (average of the three batches  $\pm$  standard deviation)

Analyte	Parameter	Result
Heavy metals (mg/kg)	Cadmium	0.01 $\pm$ 0.0
	Lead	<0.05
	Mercury	<0.04
	Arsenic	<0.03
	Tin	<0.04
Pesticides (mg/kg)	None detected	
Microbiological analysis (CFU/g)	Mesophilic aerobic bacteria	890 $\pm$ 91.65
	<i>Bacillus cereus</i>	<10
	Faecal coliforms	<10
	Total coliforms	<10
	<i>Escherichia coli</i>	<10
	<i>Enterobacteriaceae</i>	<10
	<i>Staphylococcus aureus</i>	<10
	<i>Listeria monocytogenes</i>	<10
	<i>Salmonella</i>	Not detected in 25 g
	Mould	<10
Yeast	<20	

\*Erratum/Corrigendum (16-11-2015): 0.05 instead of 0.5 and vegetables instead of algae.

## Comments

Regulation (EC) No 396/2005 lays down maximum residue limits for pesticides, including those in marine algae for a large number of those residues analysed in this case (EU, 2005a). However, since the presence of pesticides in sea water is very infrequent, mainly due to the high dilution factor, the analyses of pesticides carried out are considered to be sufficient.

The Scientific Committee takes the view that the molecular and composition studies presented accurately identify the novel food to be marketed. Likewise it considers that the applicant has provided sufficient documentation demonstrating the absence of contaminants.

## II. Effects of the production process for the novel food

The applicant describes the process for producing the novel food ingredient in detail and states that it is similar to that used to obtain *Chlorella* and *Odontella aurita*, which are microalgae authorised for human consumption. It also describes the checks carried out on the finished product and provides data to demonstrate the product's stability.

### Description of the production process

The production process described by the applicant comprises four stages: culture, harvest, drying and packaging.

#### 1. Culture

- Obtaining the inoculate. The strain from the ICMAN-CSIC algae collection is cultivated at controlled temperature, CO<sub>2</sub> atmosphere and hours of exposure to light. When the volume of the culture has increased to the required size, it is put in photobioreactors to bring it up to industrial scale.
- Industrial scale. Two types of photobioreactors, for inoculation and production, are used, both of which are located outdoors. They are closed systems insulated from the outside air to prevent contamination. They are made up of transparent tanks and tubes where the culture develops at environmental temperatures and light conditions. When the inoculate photobioreactors have produced a dense culture of microalgae, this is automatically transferred by means of a closed tube system to the production photobioreactors in which the microalgae are harvested once the required cell density has been reached.

Sterile sea water is used for the culture. The applicant has provided physical, chemical, microbiological, heavy metal and pesticide analysis of the sea water used for the culture. The analyses were conducted on three samples of water taken throughout the year. In all cases, the values obtained for the parameters were below the maximum levels permitted in Directive 98/83/EC on the quality of water for human consumption (EU, 1998).

The applicant states that, in order to maintain the culture in optimal conditions, the strain is renewed every 15 days and is renewed from the ICMAN-CSIC algae collection twice a year. Likewise it affirms that to maintain the nutritional characteristics of the strain constant, the concentration of nutrients in the culture and all the critical parameters are checked daily to guarantee the reproducibility of the process.

2. Harvest. The culture is harvested by centrifuging at 4 °C. The paste obtained is collected in plastic bags for subsequent drying.

3. Drying. The drying process is conducted at a temperature of no higher than 30 °C, producing dehydrated algae cakes.

4. Packaging. Each batch of dehydrated algae cakes is ground at a controlled temperature and vacuum-packed in 250-g bags. The bags are kept in the company's stores at a constant temperature of 20 °C.

### Production monitoring

According to the applicant, the finished product is subject to microbiological checks for: mesophilic aerobic bacteria, *Enterobacteriaceae*, *Salmonella* and *Listeria monocytogenes*. The applicant has submitted the HACCP plan which includes, apart from the microbiological parameters mentioned above, annual checks for the absence of *Clostridium botulinum* and botulinum toxin.

The applicant has declared that it has a self-monitoring system for microalgae production comprising a general hygiene plan in addition to the HACCP mentioned above. All this, it states, enables production to be monitored (raw materials, cleanliness and disinfection of the machinery used etc.) and all incidents to be recorded which may occur during the process. The applicant is certified to ISO 22000:2005 and FSSC 22000:2011.

### Stability of the finished product

The applicant has submitted a study on the stability of the dried powder and of a sauce made with the product.

- Powdered product. To demonstrate its stability the applicant has presented physico-chemical and microbiological analyses carried out two years consecutively of three batches stored at a temperature of between 23 °C and 25 °C during the entire study (Table 4).



**Table 4.** Stability of the powdered product stored at ambient temperature (the average of three batches  $\pm$  standard deviation)

	Time	0 months	12 months	24 months
Physico-chemical parameters	pH	8.16 $\pm$ 0.017	8.16 $\pm$ 0.036	8.18 $\pm$ 0.020
	Humidity (%)	6.11 $\pm$ 0.067	6.13 $\pm$ 0.064	6.14 $\pm$ 0.061
	Water activity	0.2 $\pm$ 0.001	0.2 $\pm$ 0.001	0.2 $\pm$ 0.002
	Colour/odour	Satisfactory	Satisfactory	Satisfactory
	Chlorophyll a ( $\mu$ g/mg)	11.45 $\pm$ 0.112	11.29 $\pm$ 0.051	11.22 $\pm$ 0.020
Microbiological parameters (CFU/g)	Mesophilic aerobic bacteria (30 °C)	840 $\pm$ 81.85	840 $\pm$ 75.49	740 $\pm$ 40.00
	<i>Enterobacteriaceae</i> positive lactose (30 °C)	<10	<10	<10
	<i>Listeria monocytogenes</i> (37 °C)	Not detected	Not detected	Not detected
	<i>Salmonella</i> (37 °C)	Not detected	Not detected	Not detected

- Sauce prepared with the product. The applicant has presented a stability study on a sauce prepared using potassium sorbate, salt, citric acid, xanthan gum and dried *Tetraselmis chuii* at a concentration of 20 %. None of the parameters studied (mesophilic aerobic bacteria, coliform, *Listeria monocytogenes* and *Salmonella*) were detected during microbiological analysis of a previously sterilised batch of the sauce carried out every month for a year.

### Comments

The Scientific Committee approves the description provided of the production process. The culture in photobioreactors isolated from the environment precludes external contamination and the production monitoring and hygiene systems (HACCP and the hygiene plan) laid down by the company are deemed to be adequate.

The stability studies revealed no significant variations in the product in the forms in which the applicant would like to market it during the period of observation. There are no stability data in connection with the macronutrient composition but these are not considered to be indispensable because of the low nutritional value of the product. It is recommended that periods no longer than those in the study for each type of product be placed on the label as the best-before and use-by dates.

### III. History of the organism used as a source of food

The taxonomic classification of the microalgae *Tetraselmis chuii* Butcher was described in section I. The applicant states that the microalgae *Tetraselmis chuii* is exclusively used in cultivating crustaceans and molluscs and as a primary link in the food chain for the larvae of farmed fish such as bream. The applicant therefore considers that although there are no references to direct consumption by humans, the species *Tetraselmis chuii* has been introduced indirectly into the human food chain.

Fitoplancton Marino S.L. has been producing the dried microalgae on an industrial scale since 2005 for cultivating the larvae of fish and various types of molluscs.

## Comments

The Committee has reviewed the information and has no comments on this section.

### IX. Planned intake/level of use of the novel food

Various species of microalgae are currently marketed in some countries as food supplements and ingredients in various foods (pasta, soup, bread, rice, beverages, cereals and condiments).

Consumption of microalgae, mainly *Chlorella* sp., is widespread in North America and, to demonstrate this, the applicant has provided the addresses of points of sale on the Internet and the recommended doses. It has presented a comparison of the profile of amino acids contained in the species of microalgae *Tetraselmis chuii* and *Chlorella pyrenoidosa* showing that the amino acids content is similar.

Fitoplancton Marino S.L. wishes to market the dried *Tetraselmis chuii* as a seafood flavouring condiment, to be added directly in the form of powder or incorporated in salt as an aromatic and in sauces as a taste enhancer. In view of its organoleptic characteristics, the applicant laid down a daily intake of the dried *Tetraselmis chuii* of 250 mg/day.

- Sauces. The uptake per person/day of salt with 20 % of the product would be a portion of 1.25 g. This would contain 250 mg of the microalgae. The applicant affirms that the sauce will be packaged in containers labelled with the number of portions.
- Salt. Prepared with 1 % of the dried *T. chuii*. Maximum recommended intake of salt by the World Health Organization (WHO) is 5 g/day and the envisaged consumption of the dried microalgae would be 50 mg/day (WHO, 2002).
- Powder. Direct use of the dried powder as a condiment. The envisaged intake would be 250 mg/day. The applicant states that it would package the product in single-dose containers or in larger containers for the food industry.

## Comments

The Scientific Committee considers the estimates of intake made by the applicant to be appropriate.

### XI. Nutritional information on the novel food

The applicant declares that dried *Tetraselmis chuii* is made up of entire cells without any component being fractioned or extracted, which means that it is nutritionally equivalent to its source. The novel food will be used as a seafood flavouring ingredient in salt and sauces or as a powder added to various prepared dishes such as pasta or rice dishes.

The applicant has presented analyses of the nutritional profile of three batches showing that it is made up mainly of proteins and carbohydrates and, to a lesser extent, fat. The nutrients provided by ingesting the dried *Tetraselmis chuii* at the concentration proposed by the applicant are few.

Marine algae have been described as being capable of synthesising long-chain PUFA (polyunsaturated fatty acids), some of which cannot be synthesised by plants or animals. According to the analyses provided by the applicant, most of the fats are PUFA and amongst those the most abundant are linolenic acid (C18:3) and eicosapentaenoic acid (EPA, also called timnodonic acid) (C20:5). At the intake estimated by the applicant, consumption of the product would provide 7.8 mg/day of PUFA,

4.17 mg of which would be linolenic acid, representing 0.21 % of the daily intake recommended by European Food Safety Authority (EFSA, 2009, 2010). Only the uptake of iodine might be relevant.

The applicant has presented a comparative study of the nutritional profile of rice and pasta ready-made dishes with/without seasoning of 250 mg of dried *Tetraselmis chuii* in the form of powder or salt. For portions of rice and pasta of approximately 60-80 g/day, the total amount of *Tetraselmis chuii* ingested would be 0.3 % of the total quantity consumed.

A comparative study of the nutritional profile of salt, iodised salt and salt with dried *Tetraselmis chuii* was also presented.

### Comments

The applicant has provided inductively coupled plasma mass spectrometry (ICP-MS) analyses conducted in two different laboratories. In one of them iodine was not detected in any of the three samples of the different batches analysed with a limit of detection of 54 mg/kg and in the other laboratory with a limit of detection of 0.01 mg/kg and a quantification limit of 0.019 mg/kg, values of between 12.5 and 0.45 mg/kg were detected in the four batches analysed.

Taking the highest value obtained in the analyses conducted by the latter laboratory (12.5 mg/kg) and the consumption estimated by the applicant (250 mg), the quantity of iodine ingested would be 3.1 µg/day, which would be 2.1 % of the recommended daily intake (RDI) of iodine for adults (EFSA, 2006). For a population ingesting a daily portion of sauce with 250 mg of the microalgae, 5 g of salt and a dish seasoned with 250 mg of the client product, the uptake of iodine from this novel food would be 4.6 % of the RDI for iodine.

In relation to the uptake of PUFA and taking into account the scenario described previously this may be considered as insignificant.

As mentioned above, according to the applicant, the microalgae *Chlorella* is consumed as a food supplement in the European Union, mainly because of the protein, essential amino acid, mineral and trace element content and the applicant states that there are many similarities between this and the microalgae *Tetraselmis chuii*, the dried version of which it wishes to market.

The Committee takes the view that the novel food does not provide any added nutritional value to foods but nor does it constitute nutritional liability because this microalgae would replace other microalgae of similar composition.

## XII. Microbiological information on the novel food

In the analyses presented by the applicant of the three batches of the product no pathogenic microorganisms were detected (Table 5). The mesophilic bacteria count showed high values of around  $10^3$  CFU/g. According to the bibliography submitted by the applicant these values are considered to be usual in many species of microalgae because there is a symbiotic relationship at nutrient level between microalgae and certain species of bacteria.

In Spain there are no specific regulations for the microbiological content of algae but microbiological criteria laid down in France provide for a limit of mesophiles of  $\leq 10^5$  CFU/g in the dried product (CEVA, 2012). Regulation (EC) No 2073/2005 on the microbiological criteria applicable to food products (EU,

2005b) lays down a limit of 100 CFU/g for *Listeria monocytogenes* which would be applicable if the new product is considered as a food ready for consumption.

As mentioned in section II, the applicant has provided documentation according to which the dried *Tetraselmis chuii* is manufactured following a self-monitoring system. This system is made up of a general hygiene plan and a HACCP system, both of which were reviewed by the Andalusian regional government's health department. The hygiene checks are made by an external company which periodically takes samples of the surfaces and equipment used for the production process. The company is certified to ISO 22000:2005 and FSSC 22000:2011.

**Table 5.** Microbiological analysis of dried *Tetraselmis chuii* (average of three batches  $\pm$  standard deviation)

Parameter	Dried <i>T. chuii</i> (CFU/g)
Mesophilic aerobic bacteria	890 $\pm$ 91.65
Mould	<10
Yeast	<20
Faecal coliform	<10
Total coliforms	<10
<i>Enterobacteriaceae</i>	<10
<i>Escherichia coli</i>	<10
<i>Staphylococcus aureus</i>	<10
<i>Bacillus cereus</i>	<10
<i>Listeria monocytogenes</i>	<10
<i>Salmonella</i>	Not detected/25 g
<i>Clostridium perfringens</i>	<1
Sulfite-reducing clostridia	<1

### Comments

The Scientific Committee considers the documentation presented to be adequate as the microbiological analyses do not show the presence of pathogenic organisms and the company has submitted its HACCP system and hygiene plan.

### XIII. Toxicological information on the novel food

The microalga to be marketed is used in aquaculture for the industrial cultivation of crustaceans, molluscs and fish larvae without any toxic effects having been detected. The applicant declares that the species of algae likely to produce toxins are members of seven of the 76 orders of algae microorganisms, none of which belong to the *Plantae* kingdom, a kingdom in which no toxinogen has yet been described.

The CSIC certificate is annexed mentioning that this species "neither produces nor accumulates toxins". The Australian microalgae collection, belonging to the Commonwealth Scientific and Industrial Research Organisation (CSIRO), indicates that the species *Tetraselmis chuii* is not toxic (CSIRO, 2013).

Nevertheless the applicant has conducted toxin analysis and studies of toxicity, genotoxicity and allergenicity in the dried *Tetraselmis chuii*.

### Toxin analysis

The applicant has carried out analyses on three different batches using the method of bioassay in rats for PSP and DSP toxins and liquid chromatography analysis of amnesic (ASP) toxin. The results were negative for all the toxins and batches assayed. The tests were conducted in laboratories accredited to UNE/EN ISO/IEC 17025 for the analysis of agrifood products.

### Toxicity and genotoxicity study

The applicant presented studies of acute toxicity (OECD No 423) and 90-day toxicity (OECD No 408) for dried *Tetraselmis chuii* conducted in accordance with the principles of good laboratory practice. Likewise, the applicant presented the results of a test of reverse mutagenicity on bacteria or Ames test (OECD No 471).

The value of  $DL_{50}$  obtained in the acute toxicity test was 2 500 mg/kg body weight and there were no signs of toxicity in the animals treated.

From the 90-day study on rats an NOAEL of 2 500 mg of the product/kg body weight/day was calculated. The conditions for the test were as follows: oral doses of 625, 1 667 and 2 500 mg of the product/kg/day administered in a gel to rats of both sexes for a period of 13 weeks. The dose levels were set on the basis of a previous 14-day oral palatability study conducted on the product. Throughout the test, consumption of food and water, body weight and changes in behaviour were checked and ophthalmological examinations, and biochemical and haematological analyses were carried out as indicated in the test protocol on the control animals and treated animals without any significant changes at any of the doses tested being observed. At the end of the study the animals were killed and histopathological examinations on organs and tissues of all the animals were carried out on both the controls and the animals treated. The *post-mortem* revealed that, only in the females administered 625 mg/kg/day, there was a significant increase in the weight of the heart compared with those treated with the higher dose. However, this significant difference is viewed as an isolated fact without any dose-related trend having been observed.

The Ames test was conducted on strains TA98, TA100, TA1535 and TA1537 of *Salmonella typhimurium* and strain WP2 (pKM101) of *Escherichia coli*, with and without metabolic activation, using five concentrations in a range of 4.00 to 0.05 mg/plate. In order to set the test dose, a previous cytotoxicity study was carried out on one of the strains with negative results for all the doses used.

The result was negative in all the strains and at all the concentrations used indicating that the dried *Tetraselmis chuii* does not induce any mutations and can be considered to be non-mutagenic on the basis of the tests.

## Allergenicity studies

With regard to the absence of allergenicity the applicant claims that:

- The species *Tetraselmis chuii* is similar to the species of *Chlorella* which has a history of consumption in the European Union without any allergic episodes being recorded after consumption.
- The results of analysis with allergens present in crustaceans, salmonids, engraulidae and histamine were negative. These allergens were selected by the applicant because of the marine origin of the novel food.
- The results of the analysis of allergens present in soya and gluten were negative. These allergens were selected by the applicant because the novel food may be considered a plant food.

The applicant presented analysis of the sulphite content –15 mg/kg– in the dried *Tetraselmis chuii*. Sulphites appear in natural form and the applicant indicates that it would include on the label an indication “contains sulphites” as laid down in Directive 2000/13/EC on the approximation of the laws of the Member States relating to the labelling, presentation and advertising of foodstuffs (EU, 2000).

Furthermore, the applicant presented two studies on the sensitising capacity of the product in humans (prick test and patch test). Both studies were carried out following the guidelines of the Spanish Group for Research on Contact Dermatitis and Cutaneous Allergies (GEIDAC) and the European Society for Contact Dermatitis (ESCD).

- Assessment of the sensitisation capacity type I in the Gell-Coombs classification (immunoglobulin method). For this the applicant presented a prick test on 100 healthy individuals, 50 of whom had histories of allergies. In all of them a saturated solution with a serum of the dried product was applied with histamine as a positive control and saline as a negative control. Likewise, this test was carried out on all the workers at Fitoplancton Marino, two of whom had histories of allergies. No positive reaction to the saturated solution of dried *Tetraselmis chuii* was observed in any individual.
- Assessment of the irritant and sensitisation capacity type IV in the Gell-Coombs classification. For this the applicant presented a patch test on 30 healthy volunteers to whom a saturated solution of the product was applied using standard media. No positive reaction to the product was observed in any of the individuals.

## Comments

The Scientific Committee considers the harmlessness of the novel food to have been demonstrated.

## Conclusions of the Scientific Committee

The AESAN Scientific Committee considers that the information provided gives no indication that the consumption of a species of the marine microalgae *Tetraselmis chuii* as a condiment under the conditions proposed by the applicant can produce any adverse effects on health. The Scientific Committee concludes that the novel food presented for assessment by Fitoplancton Marino S.L. meets the criteria for acceptance laid down by Regulation (EC) No 258/97 on novel foods and novel food ingredients.

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