



ORIGINAL SUBMISSION

000001



Division of Biotechnology and GRAS Notice Review  
Office of Food Additive Safety-CFSAN  
U.S. Food and Drug Administration  
5100 Paint Branch Parkway (HFS-255)  
College Park, MD 20740-3835

January 23, 2012

ATTN: Dr. Antonia Mattia, PhD

Our Reference: GRAS Notification and Exemption Claim for Certified Organic Spirulina

Dear Dr. Mattia,

AIBMR Life Sciences, Inc. has been retained as an agent by E.I.D. Parry (India) Limited, Parry Nutraceuticals Division ("the Notifier") to submit a GRAS notification to the FDA for Certified Organic Spirulina, a powdered preparation of organically grown *Arthrospira platensis* to be used as an ingredient in the enclosed specified categories of food.

Please find enclosed three copies of the notification *Notice to US Food and Drug Administration that the use of Certified Organic Spirulina (Arthrospira platensis) is Generally Recognized as Safe*. As stated in the exemption claim, the data and the information that serve as the basis for this GRAS determination will be available for review and copying at reasonable times at the office of Parry Nutraceuticals Division, E.I.D Parry (India) Ltd., "Dare House" No.234, N.S.C Bose Road, Chennai – 600 001, India; or will be sent to FDA upon request.

Parry Nutraceuticals (the notifier), has determined that Certified Organic Spirulina is Generally Regarded as Safe (GRAS), consistent with section 201 (s) of the Federal Food, Drug and Cosmetic Act. This determination has been made based on scientific procedures. Spirulina has a long history of human consumption and has been thoroughly researched in toxicological models, with no results prompting concern for safety. It has been recommended as a food for human consumption by governmental agencies. In summary, the use of Certified Organic Spirulina in the enclosed specified categories of food is exempt from the requirement of pre-market approval.

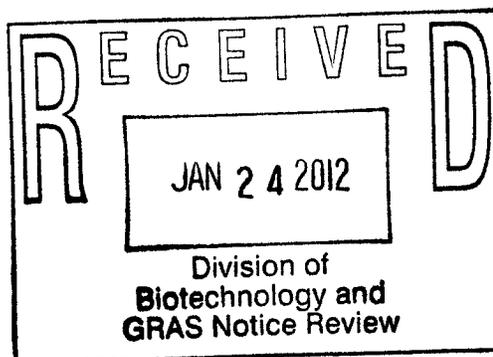
Yours sincerely,

John R. Endres  
Chief Scientific Officer  
AIBMR Life Sciences, Inc.  
john@aibmr.com

4117 SOUTH MERIDIAN  
PUYALLUP, WA 98373

(253) 286-2888 PH  
(253) 286-2451

WWW.AIBMR.COM



000002

**Notice to US Food and Drug Administration  
that the use of Certified Organic Spirulina  
(*Arthrospira platensis*) is Generally  
Recognized as Safe**

**Submitted by the Notifier:  
E.I.D. Parry (India) Limited,  
Parry Nutraceuticals Division,  
"Dare House"  
No.234, N.S.C Bose Road,  
Chennai – 600 001, India**

**Prepared by the Agent of the Notifier:**

AIBMR Life Sciences, Inc.  
4117 S Meridian  
Puyallup WA 98373

**January 20<sup>th</sup>, 2012**

**000003**

## Table of Contents

<b>GRAS Exemption Claim .....</b>	<b>3</b>
<b>Characterization .....</b>	<b>5</b>
<b>Manufacturing and Production .....</b>	<b>6</b>
Company Overview .....	6
Raw Materials .....	6
Water .....	7
Inoculation Nutrients .....	7
Pond Cultivation Nutrients .....	7
Manufacturing Overview .....	8
Cultivation .....	8
Pond Maintenance and Monitoring .....	8
Harvesting and Packaging .....	9
<b>Specifications, Batch Analysis and Quality Management .....</b>	<b>9</b>
Specifications and Batch Analyses .....	9
Residual Pesticide and Other Contaminant Analysis .....	11
Algal Toxins and Pheophorbides .....	11
Shelf-life Stability .....	12
<b>Self-limiting Levels of Use .....</b>	<b>12</b>
<b>Safety Assessment of <i>S. platensis</i> .....</b>	<b>12</b>
Acute and sub-chronic toxicity studies .....	13
<i>In vitro</i> and 6-month <i>in vivo</i> safety assessment studies of <i>S. platensis</i> .....	15
Reproductive Study of <i>S. platensis</i> in rats .....	17
<b>Safety assessment of other <i>Spirulina</i> species .....</b>	<b>18</b>
Sub-Chronic Oral Toxicity Studies .....	18
Chronic Oral Toxicity Studies .....	19
Reproductive and Developmental Toxicity Study .....	19
<b>Additional Scientific Studies .....</b>	<b>21</b>
<b>Immunological-Specific Studies .....</b>	<b>21</b>
<b>Spirulina Consumption .....</b>	<b>23</b>
History of Consumption .....	23
Previous Sales and Reported Adverse Events .....	24
Current Regulatory and International Status .....	24
<b>Information that may appear to be Inconsistent with GRAS Determination of Spirulina .....</b>	<b>26</b>
<b>Intended Use .....</b>	<b>27</b>
Categories of Food .....	27
Estimated Daily Intake (EDI)—Exposure .....	28
<b>General Recognition .....</b>	<b>29</b>
<b>Basis for the GRAS Determination .....</b>	<b>30</b>
<b>References .....</b>	<b>31</b>

## GRAS Exemption Claim

E.I.D. Parry (India) Limited, Parry Nutraceuticals Division (the notifier), in consultation with an independent panel of experts qualified by scientific training and experience to evaluate the safety of ingredients intended for use in food, has determined that Certified Organic Spirulina is Generally Recognized as Safe (GRAS) for its intended use, consistent with section 201 (s) of the Federal Food, Drug and Cosmetic Act. The determination has been made based on scientific procedures, and therefore the use of Organic Spirulina for its intended use is exempt from the requirement of pre-market approval.

(b) (6)

20<sup>th</sup> January 2012

Dr. L. Rajendran  
Head, Quality Assurance  
E.I.D. Parry (India) Limited  
Parry Nutraceuticals Division

Date

### Name and Address of the Notifier

#### Notifier

E.I.D. Parry (India) Limited,  
Parry Nutraceuticals Division,  
"Dare House"  
No.234, N.S.C Bose Road,  
Chennai – 600 001, India

#### Agent of the Notifier

John R. Endres, ND  
Chief Scientific Officer  
AIBMR Life Sciences, Inc.  
4117 S. Meridian  
Puyallup, WA 98373  
Tel: (253) 286-2888 x101; Fax: (253) 286-2451  
john@aibmr.com

### Common or Usual Name

Certified Organic Spirulina (a powdered preparation of organically grown *Arthrospira platensis*)

### Conditions of Use

Certified Organic Spirulina is intended for use at levels of 0.5–3 grams per serving, as an ingredient in the following food categories; *beverages and beverage*

*bases* (nonalcoholic, including only special or spiced teas, soft drinks, coffee substitutes, and fruit and vegetable flavored gelatin drinks); *breakfast cereals* (including ready-to-eat and instant and regular hot cereals); *fresh fruits and fruit juices* (including only raw fruits, citrus, melons, and berries, and home-prepared "ades" and punches made therefrom); *frozen dairy desserts and mixes* (including ice cream, ice milks, sherbets, and other frozen dairy desserts and specialties); *grain products and pastas* (including macaroni and noodle products, rice dishes, and frozen multicourse meals, without meat or vegetables); *milk products* (including flavored milks and milk drinks, dry milks, toppings, snack dips, spreads, weight control milk beverages, and other milk origin products); *plant protein products* (including the National Academy of Sciences/National Research Council "reconstituted vegetable protein" category, and meat, poultry, and fish substitutes, analogs, and extender products made from plant proteins); *processed fruits and fruit juices* (including all commercially processed fruits, citrus, berries, and mixtures; salads, juices and juice punches, concentrates, dilutions, "ades", and drink substitutes made therefrom); *processed vegetables and vegetable juices* (including all commercially processed vegetables, vegetable dishes, frozen multicourse vegetable meals, and vegetable juices and blends) *snack foods* (including chips, pretzels, and other novelty snacks); *soft candy* (including candy bars, chocolates, fudge, mints, and other chewy or nougat candies); and *soups and soup mixes* (including commercially prepared meat, fish, poultry (at levels that fall within FDA jurisdiction), vegetable, and combination soups and soup mixes).

#### **Basis for GRAS determination**

Scientific procedures are the basis for this GRAS determination.

#### **Data/Information Availability Statement**

The data and the information that serve as the basis for this GRAS determination will be available for review and copying at reasonable times at the office of Parry Nutraceuticals Division, E.I.D Parry (India) Ltd., "Dare House" No.234, N.S.C Bose Road, Chennai – 600 001, India; or will be sent to FDA upon request.

## Characterization

Certified Organic Spirulina is Parry Nutraceuticals' spray dried powder consisting of whole, dry cells of *Arthrospira* (*Spirulina*) *platensis*; a cyanobacterium commonly known by its traditional name "Spirulina" (Vonshak 1997). All edible forms of Spirulina that are under commercial cultivation belong to the Genus *Arthrospira* (Tomaselli et al. 1996). Parry's source strain for Spirulina culture was obtained from the Indian Agricultural Research Institute (IARI), New Delhi culture collection. The IARI strain has been taxonomically identified and verified as non-GMO *A. platensis*. This species and strain of filamentous cyanobacteria is originally from Lake Chad in Africa. Other *Arthrospira* species and strains are found in other tropical and sub-tropical water bodies in Africa, Asia, and South America. Since *Arthrospira* is commonly marketed with the trade name Spirulina, and since Spirulina is the name used commercially, this report uses the two interchangeably with the understanding that Certified Organic Spirulina is *A. platensis* and other commercially produced Spirulina are strains of *Arthrospira*.

*A. platensis* is one of three commonly cultivated and investigated *Arthrospira* cyanobacterium species. The other two are *A. maxima* and *A. fusiformis*. All three are frequently referred to by the traditional names of *Spirulina platensis*, *Spirulina maxima*, and *Spirulina fusiformis*. Microscopically, they appear as blue-green filaments composed of cylindrical cells arranged in unbranched, helicoidal trichomes. The main morphological feature of the genus is the open left hand helix arrangement along the entire length of the multicellular trichomes. Spirulina species can be distinguished based on documented morphological features, although different environmental, growth and stress conditions may elicit morphological elasticity variations among these species. (Vonshak 1997); (Desikachary et al. 1996); (Garrity 2005). *Spirulina platensis* and *Spirulina maxima* are the two common *Spirulina* species used in modern nutritional supplements (Khan 2005).

## Chemical Composition

Certified Organic Spirulina is a fine, uniform powder, blue-green to green in color, with a mild odor and taste. It contains a high nutritional density, including a wide range and abundance of macro- and micronutrients, as well as phytochemicals. In terms of macronutrients, Certified Organic Spirulina contains high levels of protein (56–69%) with a balanced amino acid profile, 15–25% carbohydrates (mainly glucose), and 5–6% lipids, mainly as polyunsaturated fatty acids with a high ratio of gamma-linolenic acid (GLA). The micronutrient composition features relatively high concentrations of B-vitamins, especially cyanocobalamin (0.05–0.20 mg/100g). Spirulina's phytonutrient composition includes phycocyanins (phycobiliproteins involved in light harvesting reactions), carotenes (including beta-carotene), chlorophyll, and xanthophylls. Batches of Certified Organic Spirulina are routinely assayed in Parry Nutraceuticals' Quality Assurance Lab for protein, moisture, total ash (minerals), bulk density, light filth, algal (microcystin) toxins and microbes in addition to the phytopigments listed in Table 1.

## **Organic Certification**

In addition to USDA National Organic Program (NOP) organic standards, Parry Nutraceuticals cultivates Certified Organic Spirulina under a number of other major international organic standards: Naturland (Germany), ECOCERT certification (France), and OCIA -USA (Organic Crop Improvement Association). These accredited organizations are world leaders in the certification of organic products, and their certifications guarantee conformity and traceability to American, European, or other standards. The product is also certified Kosher (Star K) and Halal.

Parry Nutraceuticals' Spirulina is certified by USP under the USP Ingredient Verification Program—meeting USP specifications for GMP, manufacturing, quality control documentation, and label claims.

## **Manufacturing and Production**

### **Company Overview**

Parry Nutraceuticals has a twenty-year history of research and development in microalgal biotechnology. The company manufactures and produces USDA National Organic Program Certified Organic Spirulina at their production facility located in Oonaiyur, in South India. The 120-acre facility is located in a remote area where temperatures and climate are conducive to Spirulina production (21–39° Celsius, intense sunlight, minimal precipitation), where there is no ground water contamination, and where there is no other agricultural activity—eliminating the possibility of artificial fertilizer or pesticide contamination. The mass production of Certified Organic Spirulina utilizes the open-pond, or raceway pond system which was developed in the 1950s and is widely used for outdoor mass cultivation of photosynthetic microorganisms. The production facility at Oonaiyur has twenty-nine acres of raceway ponds, including production ponds and two evaporation ponds with a capacity of 19,000 cubic meters. The facility also houses two pump/processing houses, an effluent treatment plant to meet the Pollution Control Board's requirements, a granulation and packing facility, a finished product warehouse, a raw materials storehouse, and a laboratory.

All of Parry Nutraceuticals' products comply with all applicable regulations and directives of the USA, EU and other nations on non-genetically modified, non-allergen, non-irradiation, transmissibles and bovine spongiform encephalopathy (TSE/BSE), dioxins, polychlorinated biphenyl (PCB), and traceability. Parry Nutraceuticals' products are currently exported to over thirty-five countries in North America, South East Asia and Western Europe, including the USA.

### **Raw Materials**

Along with the Spirulina inoculum, the raw materials used in the manufacturing of Certified Organic Spirulina include water, inoculation nutrients, and pond cultivation nutrients. The inoculation and pond cultivation nutrients, discussed below, consist of sea salt and organic nutrients of plant origin. The materials are purchased from approved vendors. All materials must arrive with supporting

documents. Incoming raw materials are inspected, sampled, and stored in designated raw material storage houses. Random samples of 2% of the number of containers received are sent to Parry Nutraceuticals' in-house laboratory or approved outside laboratories where they are tested and verified by designated authorities.

### **Water**

The water used at Parry Nutraceuticals for the cultivation and processing of Spirulina is drawn from a series of bore wells within the 120-acre facility. The bore well water is softened to less than 100 ppm hardness for use in the production process and run through PVC piping. After the algal biomass has been pumped from the ponds and pre-filtered, water (with no additives or processing aids) is also used for washing the harvested algal biomass in the concentrator.

Water samples are tested daily for physical appearance and hardness and monthly for microbiological contamination. Water is also tested once every six months for heavy metals. A 100–250 mL random sample is sent to an accredited laboratory identified by Parry Nutraceuticals and analyzed for arsenic, cadmium, lead and mercury per AOAC 2000 protocols. Acceptable limits are per the specifications determined by Parry Nutraceuticals.

### **Inoculation Nutrients**

Raw materials are added at two stages of production: the initial (indoor) inoculation stage and the outdoor raceway pond cultivation stage (see "Production" section below).

The raw materials used for the initial inoculation stage are added to distilled water and the Spirulina inoculum. These materials constitute the inoculation medium, known as Modified Zarrouk Medium. The nutrients for preparing the Modified Zarrouk Medium include an organic nitrogen source (5 g/L) and sea salt (1 g/L).

### **Pond Cultivation Nutrients**

The raw materials used for the outdoor cultivation of Certified Organic Spirulina consist of organic vegetable-origin fertilizers (normally code named as "PN" for Plant Nutrients) water, and sea salt. The PN utilized are certified organic as per USDA and Ecocert organic standards and are certified for use in organic agriculture. They are added to the production ponds on a need basis (see further details below). Varying amounts are added to the ponds when required, in order to optimize the biomass density and health and to prevent the growth of contaminants.

Sea salt is also added to the production ponds on a need basis. The sea salt is purchased from an approved vendor and must demonstrate purity. An approved laboratory is used to analyze the moisture content, sodium chloride content, other soluble and insoluble matter, and aluminum silicate content. The

sea salt is also analyzed for heavy metals, as described above for all raw materials.

### **Manufacturing Overview**

Parry Nutraceuticals cultivation and production process is certified under ISO 9001 (quality management systems), ISO 14001 (environmental management systems), and ISO 22000 (food safety management systems). Quality systems have been established to ensure that all of the algal products produced meet the requirements established in food GMP (CFR part 110). The facility also meets FDA GMP dietary supplement standards as demonstrated by Parry Nutraceuticals' United States Pharmacopeia (USP) certification.

Parry Nutraceuticals' production process follows the open raceway pond design which is widely used for the outdoor mass cultivation of photosynthetic microorganisms. This process involves indoor and then semi-outdoor cultivation of the *Spirulina* culture, followed by outdoor pond cultivation, harvesting, washing, drying, packing, warehousing and shipping. The entire production process is designed and carefully monitored to encourage *A. platensis* growth and prevent contamination.

### **Cultivation**

The initial indoor cultivation involves growing *Spirulina* culture in sterile flasks under artificial lights in the prescribed organic medium, which consists of distilled water and the raw materials discussed previously. It is then progressively cultured and sub-cultured, first in flasks and then in larger plastic tubs. The outdoor cultivation involves the inoculation of tub cultures into a seed pond, followed by the progressive inoculation of other seed ponds until a certain level of biomass is met, at which time this culture is used to inoculate ½-acre production ponds. This organic production pond is then sub-cultured again into ponds earmarked for *Spirulina* production. The production ponds may contain varying volumes of culture depending on the pond depth. The ponds are agitated by paddle wheels in order to facilitate light distribution and nutrient distribution, minimize self-shading due to buoyancy, and maintain a uniform temperature. Nutrients (as listed under Raw Materials), and soft water are added to the ponds, which are agitated daily.

### **Pond Maintenance and Monitoring**

The maintenance and monitoring of the ponds involves daily culture medium testing (sample collection, pond depth maintenance, optical density, and pH), microscopy, chemical analysis, and meteorological notation (temperature, sunshine duration, and rainfall). After agitating for two hours, a 50 mL culture sample is collected daily from a marked place in all ponds. From the sample, optical density is measured using a spectrophotometer in order to estimate biomass density. (The timing of the culturing and sub-culturing process, in which the *Spirulina* culture is inoculated in a series of progressively larger tubs and ponds, is based on the optical density.) The pH is measured using a pH meter. Microscopy is also performed daily. A drop of culture from each pond is examined microscopically for contamination by other algae, rotifers,

zooplanktons, motile flagellates, and precipitates. In addition, the health and size of the trichomes (*Arthrospira's* distinguishing morphological feature), are determined daily via microscopy. Chemical analysis of the culture medium is done on an as-needed basis and includes testing for nitrogen, phosphorous, sulfur, bicarbonate, and carbonate using standard methods. Spirulina is selected for harvest based on the pond's biomass levels, the culture medium testing, and the microscopy results.

### Harvesting and Packaging

The harvesting, washing, and drying of the Spirulina begins with cleaning all process lines, sumps, tanks, machines, pipes, other equipment, and rooms. The culture is then pumped from the ponds to the process building where it is pre-filtered, and then sent through a concentrator for washing with water. The collected algal biomass is stored in tanks and cooled by a water-cooling system until ready for drying. It is then spray dried meeting all food quality standards. Finally, it is collected and packaged under nitrogen or vacuum packed in multi-layer food-grade poly bags. The bags are stored in carton boxes that have been tagged with a batch number. The cartons are stacked in a place designated for Organic Spirulina in a separate finished product warehouse kept free of moisture and contamination.

## Specifications, Batch Analysis and Quality Management

### Specifications and Batch Analyses

Production consistency is tested in production lots. As shown in Table 1 below, four non-sequential batches were reasonably consistent and met all of the product specifications for physical/general composition, phytopigments, heavy metals, microbial analyses and absence of microcystin.

Table 1: Specifications and Batch Analysis of Organic Spirulina

Parameter	Specification	Method	Lot Numbers			
			PS-0424-VNK/10-11	PS-0425-VNK/10-11	PS-0531-VNK/10-11	PS-0532-VNK/10-11
<b>Physical Properties/General Composition</b>						
Protein (% dry wt)	56-69	AOAC 978.04 16th Edition	62.14	62.20	62.05	62.10
Moisture (% dry wt)	2.5-6.0	AOAC 934.01 16th Edition	4.20	4.15	4.2	4.15
Total Ash (Minerals) (% dry wt)	6.0-9.0	AOAC 930.05 16th Edition	6.99	7.02	6.90	7.10
Bulk Density (g/cc)	0.62-0.85	C.Vijayaraghavan (1995). A Practical handbook of physical pharmaceuticals, 1995.	0.769	0.769	0.769	0.769
<b>Phytopigments</b>						

<b>Total Carotenoids (mg/100 g dry wt)</b>	400–650	Strickland and Parsons (1972). A Practical Handbook of Seawater Analysis	425	434	426	420
<b>Beta-Carotene (mg/100 g dry wt)</b>	150–250	Ranganna S. (1986). Handbook of analysis and QC for fruit & veg. Products.	162	165	164	162
<b>Xanthophylls (mg/100 g dry wt)</b>	250–470	In house method	263	269	262	258
<b>Crude Phycocyanin (% dry wt)</b>	15–19	Boussiba, S, Arch. Microbiol, 120:155 – 159, 1979	16.57	16.62	16.44	16.37
<b>Chlorophyll-a (% dry wt)</b>	1.23–1.67	Vonshak, A. 1997. Spirulina platensis (Arthrospira) physiology, cell biology and biotechnology.	1.48	1.49	1.42	1.39
<b>Total Pheophorbide (% dry wt)</b>	≤ 0.12	A. Test method for Spirulina by JHFA, Environmental Food Number 99 (1981).	0.019	0.021	0.021	0.022
<b>Existing Pheophorbide (% dry wt)</b>	≤ 0.08	B. Seward R. Brown. Absorption Coefficients of Chlorophyll Derivatives. J.Fish. Res. BdCanada 25 (3) 523 – 540, 1968	0.017	0.019	0.020	0.020
<b>Light Filth (pieces/50g)</b>	≤ 50	Richard Gorham, J. (1977). Training manual for Analytical Entomology in the food industry. FDA Tech. Bulletin No: 2	5	6	7	7
<b>Heavy Metals</b>						
<b>Lead (ppm)</b>	≤ 0.2	AOAC 18 <sup>th</sup> Edition: 2006 by ICPMS	0.108	0.0938	0.137	0.135
<b>Arsenic (ppm)</b>	≤ 0.5		0.300	0.270	0.252	0.252
<b>Cadmium (ppm)</b>	≤ 0.2		<0.0100	0.0112	0.0176	0.0186
<b>Mercury (ppm)</b>	≤ 0.05		<0.0100	<0.0100	<0.0100	<0.0100
<b>Microbials</b>						
<b>Standard Plate Count (cfu/g)</b>	≤ 50,000	Bacteriological Analytical manual 8 <sup>th</sup> Edn, AOAC, USFDA, 1995.	8000	8200	8500	8200
<b>Yeast and Mold (cfu/g)</b>	Not more than 100		30	25	30	30
<b>Coloforms (Enterobacteriaceae) (/25g)</b>	Negative		Negative	Negative	Negative	Negative
<b>E. Coli (/25g)</b>	Negative		Negative	Negative	Negative	Negative
<b>Salmonella (/25g)</b>	Negative		Negative	Negative	Negative	Negative
<b>Staphylococci (/25g)</b>	Negative	Negative	Negative	Negative	Negative	
<b>Algal Toxin</b>						
<b>Microcystin (ppb/3g—DL: 0.5 ppb)</b>	Not Detectable	Lawrence Et Al.; Journal of AOAC International Vol. 84, No.4, 2001	Complies	Complies	Complies	Complies

BEST ORIGINAL COPY

### **Residual Pesticide and Other Contaminant Analysis**

Routine analysis of Certified Organic Spirulina powder for pesticides, aflatoxins, ochratoxin A, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and organochlorine and organophosphorous pesticides is carried out once per year. The production facility is located in a remote area where there is no other agricultural activity, eliminating the risk of artificial fertilizer or pesticide contamination. A random sample is sent to accredited laboratories identified by Parry Nutraceuticals. The compounds tested for by SGS laboratory in the three batches reviewed for this notification (batch numbers PS-0004-CNK/11-12, PS-0005-CNK/11-12 and PS-0006-CNK/11-12) include:

- Aflatoxins B1, B2, G1 and G2 (AOAC 18<sup>th</sup> edition 2006 using HPLC—detection limit 0.5–1.0 ppb)
- Ochratoxin A (AOAC 18<sup>th</sup> edition 2006—detection limit 2.5 ppb)
- Polyaromatic hydrocarbons (solvent extraction using GC/MS—detection limit 0.01 mg/kg)
- Polychlorinated biphenyl (AOAC 18<sup>th</sup> edition 2006 using GC-MS/LC-MS MS—detection limit 0.01 mg/kg)
- Organochlorine pesticides (AOAC 18<sup>th</sup> edition 2006 by GC-MS/LC-MS MS—detection limit 0.01 mg/kg)
- Organophosphorous pesticides (AOAC 18<sup>th</sup> edition 2006 by GC-MS/LC-MS MS—detection limit 0.01 mg/kg)

All compounds tested for, in all batches reviewed, were either not detectable or fell below the limits of detection for the specified assay.

### **Algal Toxins and Pheophorbides**

Some cyanobacteria can produce hepatotoxins or neurotoxins. For example, *Microcystis aeruginosa* produces microcystins, which are potent hepatotoxins and probable tumor promoters. To date, there is no report of any cyanobacterial toxins above specified limits in *Arthrospira* species, and their discovery is considered very unlikely to occur during monoculture of Spirulina in properly controlled and managed systems (Gershwin et al. 2008). Despite the fact that *A. platensis* is not considered a toxigenic cyanobacteria species and that cultivation occurs in controlled ponds rather than natural lakes, Parry routinely tests finished products for microcystins and **has never detected** this toxin.

Pheophorbides are phototoxic chlorophyll catabolites formed when a chlorophyll molecule loses its magnesium atom and phytol residue, which occurs with acidity and chlorophyllase activity. It is associated in humans with photosensitive dermatitis (Endo et al. 1982). Although no cases of Spirulina-induced photodermatitis have ever been reported or published, Parry Nutraceuticals routinely tests finished Organic Spirulina Powder for the presence of existing and total pheophorbides (see Table 3). Total pheophorbides are calculated in the same way as existing pheophorbides are calculated, with the addition of a three-hour incubation period to promote the conversion.

### **Shelf-life Stability**

An accelerated nine-month shelf-life stability study was performed to assess the stability of Certified Organic Spirulina. Key parameters tested were moisture, total carotenoids, beta-carotene, xanthophylls, crude phycocyanin, and chlorophyll-a. Microbial parameters were also tested, including yeast and mold, and a standard plate count. Tests were conducted monthly on batch number C602-NNK. Storage temperature was held at  $40^{\circ} \pm 2^{\circ}$  Celsius and relative humidity was maintained at  $75\% \pm 5\%$ . Analytical methods applied and specifications used were those used for routine batch release. No adverse changes in stability were observed for the 9-month period and all parameters tested met the specifications at all time points, indicating Certified Organic Spirulina Powder is stable at  $40^{\circ}$  Celsius and 75% relative humidity for 9 months. Because this was an accelerated study, it was concluded that the product is likely to be stable for a period of three years under the recommended storage conditions.

Additionally, a real-time shelf-life study was performed, beginning July 2009. The ingredient was stored in a food grade aluminum pouch with N<sub>2</sub> flushing, inside a box with stretch wrapping and tied with pop tape—stored at ambient temperature. The last measurement was performed on April 2011 (after 36 months). The sample will continue to be tested to determine the ultimate shelf-life. After 36 months, parameters including moisture, total carotenoids (mg/100 g), beta carotene (mg/100 g), crude phycocyanin (%), chlorophyll a (%), standard plate microbial count and yeast and mold (cfu/g) still fell within specifications for the product. Xanthophyll levels (mg/100 g) decreased just slightly below specifications; measuring 249.8 mg/100 g after 12 months (specification 250–470 mg/100 g) and 243.9 mg/100 g after 36 months. This particular batch began with a xanthophyll level on the low end of the specification at the baseline measurement (252.7 mg/100 g), and some analytical variation is expected during the testing process. Additionally, 100 g is much higher than the expected serving size; hence the slightly decreased measurement on this larger scale would reflect a minimal change at the serving size level.

### **Self-limiting Levels of Use**

There are no properties of Certified Organic Spirulina that result in self-limiting levels of use.

### **Safety Assessment of *S. platensis***

A number of investigators have studied the safety of *S. platensis* in laboratory animals. The types of studies that have been performed are comprehensive from a safety perspective, including acute, sub-chronic, chronic and reproductive studies. These studies are pivotal in determining the safety of *S. platensis* for human consumption. The pertinent studies are detailed in the section below.

## **Acute and sub-chronic toxicity studies of fresh and dried *S. platensis* by Hutadilok-Towatana and co-workers in 2008**

Hutadilok-Towatana and co-workers investigated the potential toxicity of *S. platensis* exposure in male Swiss mice in a 7-day acute oral toxicity study and in male and female Spargue-Dawley rats in a 12-week sub-chronic toxicity study (Hutadilok-Towatana et al. 2008). Fresh *S. platensis* was obtained from Yord Thong 2001 Pty. Ltd. (Songkhla, Thailand), and dried *S. platensis* was prepared by drying the fresh algae at 55–60°C for 24 hours. The dried algae were then sorted in sterile containers at 4°C in. The purpose of these two studies was to assess the safety of consuming *S. platensis*.

### **7-Day Acute Oral Toxicity Study**

Swiss male mice, 30–36 g weight range, were obtained from the Animal House Facility Unit, Faculty Science, Prince of Songkla University, (Thailand) and subjected to a short-term high-dose acute oral toxicity study. The mice received either 30 g/kg/bw fresh *S. platensis* or 10 g/kg/bw dried *S. platensis* daily for 7-days. At the end of the study, the mice were sacrificed and their internal organs examined for gross pathological abnormalities.

Upon organ examination, no *S. platensis* treatment-related pathological abnormalities were observed. The authors stated that the absence of any toxicological abnormalities at such high *S. platensis* dose levels demonstrates its safety.

### **12-Week Sub-chronic Toxicity Study**

Male and female Spargue-Dawley rats, aged 5 weeks, obtained from the National Experimental Animal Center (Nakhon Pathon, Thailand) were subjected to toxicity studies for long-term exposure to *S. platensis*. The animals were randomly divided into four groups per sex with six animals per group (total of 24 animals per sex) and housed in stainless steel cages, six animals of the same sex per cage, at 25 ± 2°C and 50–60% relative humidity. The animals received a one-week acclimation period prior to the treatment. The animals received C.P. Mice Feed, Charoen Phokphand Group (Bangkok, Thailand), and water was available *ad libitum*. In this study both fresh and dried *S. platensis* were orally administered by gavage at 5 mL/kg/ bw. Fresh *S. platensis* was orally administered at 300, 600 and 1,200 mg/kg/ bw, while dried was orally administered at 30, 60 and 120 mg/kg/ bw. Rats in the control groups received only water, 5 mL/kg/ bw.

The animals were weighed prior to the start of the study (150–300 g starting weight average) and then twice weekly for the duration of the treatment period. Daily examinations focused on behavioral changes, general clinical state of the animals, as well as food and water uptake. Blood samples were collected every four weeks for hematology and biochemical analysis. At the end of the 12-week study the animals were sacrificed for autopsy examination to identify any macroscopic abnormalities. At the necropsy examination several organs

including the heart, liver, spleen and kidneys were weighed and examined for abnormalities.

During the daily examinations no abnormal clinical, physical or behavioral changes were observed in any of the animals in any of the treatment groups. Furthermore, daily water and food consumption for animals in all the groups, including the control groups, were similar. Animals' excrement examinations revealed no signs of gastrointestinal abnormalities, and body weight measurements for animals in the treatment groups were also consistent to those of the control groups. Male rats did have a higher average growth rate compared to female rats. However, no significant differences in body weight gains, or final weight were observed in animals in any of the treatment groups compared to those in the control groups. The authors stated that a lack in weight variation is an indication that a *S. platensis* supplemented-diet did not adjust the "protein, carbohydrates and fat utilization in the rats." The authors further stated that this result is consistent with a previous study in which mice were fed *S. maxima* (Salazar et al. 1998).

Clinical chemistry results also indicated no *S. platensis*-related abnormalities, and the authors pointed out that no differences were observed in the *S. platensis* treated animals' blood lipid profiles compared to the controls. The authors mentioned that although they observed a difference between the initial and final total cholesterol and LDL-C data for females in the treatment groups, they regarded these as *variation effects* and not treatment-related effects. Additionally, although *S. platensis* is high in potassium, no abnormal potassium ion levels were observed in any of the animals.

Hematological examinations were conducted on the fresh *S. platensis* treatment groups to investigate its intravascular effects. Slight increases in hematocrit values were noted with treatment, but were overall not dose related. The white blood cell counts for male and female rats were similar to those of the control animals. At treatment termination, week 12, a decrease in lymphocyte ratios in all the *S. platensis* treated animals were observed, but coincided with an increase in neutrophil and monocyte values. The increase in neutrophil values was especially prominent in the 600 and 1,200 mg/kg/bw groups. These values were 0.33, 2.00, 10.80 and 11.50% in female rats, and 1.33, 2.20, 6.60 and 15.33% in males rats for the 0, 300, 600 and 1,200 mg/kg/bw dose levels respectively. This effect was accompanied by the gradual decrease in lymphocytes, and was only seen during week 12. The authors concluded that long-term fresh *S. platensis* exposure could result in a dose-dependant neutrophil count elevation. In response to this observation the authors stated that although an increase in neutrophil count can be associated with a response to inflammation, that upon terminal examinations no signs of infection or inflammation were detected. They concluded that the increase in neutrophil count is therefore not a result of underlying inflammation or infection.

A slight variation in monocytes values was observed during the 12-week period, but the final values for the treatment group animals were similar to those of the controls. However, males receiving a 300 mg/kg bw *S. platensis* dose had significantly elevated monocyte values (12.60%) compared to male rats in the other groups (3.50, 3.20 and 5.17% for the control, 600 and 1,200 mg/kg dose

groups respectively). The authors stated that five male rats in the 300 mg/kg/bw had elevated monocyte values, however the cause of this random event was not established. Additionally, no dose dependant trend was observed. The authors stated that the increase in monocytes could be in response to trauma caused by "daily oral gavage and monthly blood sampling." According to this 2008 study by Hutadilok-Towatana and co-workers the hematological results described above have never been reported before and require additional investigation.

Upon necropsy, no macroscopic organ abnormalities were observed for animals receiving fresh or dried *S. platensis*. The results also revealed no organ weight or organ morphological changes. Additionally, no gastrointestinal abnormalities, such as lesions, resulted from the long-term exposure to *S. platensis*—fresh or dried. According to the pathological examinations, rats receiving *S. platensis* did not exhibit any toxic effects or organ abnormalities as a result of exposure to the algae. Overall, the authors concluded that short and long-term consumptions of *S. platensis* did not produce any adverse effects in experimental animals.

#### ***In vitro* and 6-month *in vivo* safety assessment studies of *S. platensis* by Yang and co-workers in 2011**

Yang and co-workers assessed the safety of *S. platensis* (and *Nostoc commune var. sphaeroides* Kützing) by analysis of the algal toxins commonly present in many blue-green algae, carcinoma cell line cytotoxicity measurements, and mice plasma and liver examinations (Yang et al. 2011). Herein, only the assessment of *S. platensis* will be discussed, as it is the subject of this GRAS notification. The microcystin (cyanotoxin) content of *S. platensis* was determined to identify possible cyanotoxins.

#### ***In vitro* assessment of *S. platensis***

The microcystin content of *S. platensis* was measured in triplicate (three batches) by extracting powdered *S. platensis* with hot water, and analyzing the prepared extract by liquid chromatography tandem mass spectrometry. The objective was to screen the algae extract for potential cyanotoxin commonly associated with certain blue-green algae.

The extracts were screened for four major microcystins (MC), namely MC-LA, MC-RR, MC-LW and MC-LR. However, none of these were detected in any of the three *S. platensis* samples. The results support that *S. platensis* is not a toxin-producing algae.

#### **Cytotoxicity of *S. platensis***

Cultured human hepatocellular carcinoma cells (HepG2) were incubated with extracted *S. platensis* fractions (from hexane, chloroform, methanol and water) for 24 hours at 0–500 µg/mL extract concentrations. Cell viability was then measured using a Cell Counting Kit-8 (Dojindo Inc. Rockville, MD). Three different batches of *S. platensis* were examined.

Although the cell viability decreased slightly, with increased extract concentrations, the cell viability remained at approximately 80% even at 500

$\mu\text{g}/\text{mL}$  concentrations. This *in vitro* assay supports the safety of *S. platensis* with no toxicological effects.

#### **6-Month *in vivo* assessment (oral toxicity study) of *S. platensis***

Male and female mice (C57BL/6J), aged 4 weeks, were obtained from Jackson Laboratory (Bar Harbor, Maine, USA). The animals were housed in polycarbonate cages, with 8 animals of the same sex group per cage, with 12 h light-dark cycles. The animals were randomly assigned to one of three *S. platensis* dose levels at 0%, 2.5% and 5% by weight of the diet, with  $n=8$ . In this study, powdered *S. platensis* was incorporated into an AIN-93G/M diet. During the 6-month study the mice were monitored for mortality, morbidity and behavior. The animals were weighed biweekly, and blood samples were collected at 2, 4 and 6 months. At the end of the study the animals were euthanized and livers were weighed. Animal blood samples were analyzed for alanine aminotransferase (ALT) and aspartate aminotransferase (AST) to assess liver and tissue damage as a result of the *S. platensis* supplemented diets.

During the 6-month treatment period no deaths, illness or behavior related changes were observed in any of the animals (in all treatment groups). Additionally, no significant weight changes were observed in any animals when compared to the control mice. All the animals had normal growth patterns throughout the treatment period. Hence, the long-term (up to six months in this particular study) exposure to 2.5% and 5% *S. platensis* supplemented diets did not result in any observed adverse side effects.

Plasma ALT levels at all dose levels for both sexes were consistent with that of the control animals, with the exception of males in the 5% *S. platensis* group which had lower ALT plasma levels at the 2-month examination period. More importantly, there were no elevated ALT levels detected for either male or female mice on a *S. platensis* supplement diet during the duration of the study. AST levels for male rats, in both the 2.5% and 5% *S. platensis* diet supplement groups were significantly lower at the 6-month sample period. Female mice on the 5% *S. platensis* diet also showed a decrease in AST levels towards the end of the treatment period. The authors pointed out that both male and female mice in the control groups had elevated plasma AST levels (a two-fold increase) by the end of the study. One hypothesis by the authors as to why this AST level fluctuation occurred is that the Spirulina may have prevented age-related tissue damage.

Animal liver samples were subjected to histopathological examinations at termination of the study. The authors mentioned that all mice, regardless of sex and diet, exhibited "mild to moderate lipidoses" at the end of the study. However, no liver damage or signs of hepatitis were detected in any of the animals.

This *in vivo* assessment of *S. platensis* further strengthens the case that the algae do not cause any adverse effects. Furthermore, *S. platensis* can be ingested for an extended period of time without any noticeable liver or tissue damage.

### **Reproductive Study of *S. platensis* in rats.**

In 1993 Kapoor and Mehta investigated the effects of five diets consisting of 22% protein (Kapoor et al. 1993). The diets included:

- (1) Casein (300 g/kg)
- (2) *S. platensis* (480 g/kg)
- (3) Wheat gluten (374 g/kg)
- (4) *S. platensis* + wheat gluten (240 + 187 g/kg)
- (5) *S. platensis* – without additional vitamins and minerals (480 g/kg)

Diets 1–4 were supplemented with vitamins and minerals. In this study Albino rats, obtained from Disease and Germ Free Animal House of Haryana Agricultural University (Hisar, India), were housed in polypropylene cages at 22–24°C with 12 h light-dark cycles.

During the mating period two female rats were housed with 1 male rat. Female rats were examined daily and day 0 of the study occurred when sperm was detected in the vaginal smears. The pregnant female rats were housed individually and received one of the 5 feeding regimes, with 10 pregnant female rats in each group (total of 50 pregnant female rats). The animals were monitored throughout the pregnancy until the pups were delivered.

Daily food uptake for pregnant rats was similar in all the groups, with the exception of rats on the *S. platensis* diet devoid of minerals and vitamins, which had significantly lower food intake. The highest pregnancy weight gain was recorded for rats on the *S. platensis* + wheat gluten diet. Rats on the wheat gluten diet had the lowest pregnancy weight gain. The weight gain values were 95.2, 96.2, 51.6, 105.2 and 81.0 g for rats on diets 1–5. The number of pups born in groups 1–5 was 48, 63, 49, 65, and 57, respectively (each group had 10 female rats). The authors stated that the litter sizes were significantly less for females on non-*S. platensis* diets. The total litter weight was 49.6, 56.6, 42.3, 63.0 and 52.2 g for groups 1–5, respectively. All females on the *S. platensis* diets had higher litter weights. The authors mentioned that the high vitamin E content of *S. platensis* may have played a role in the litter sizes. The authors also pointed out that the higher litter weights corresponded to larger litter sizes for *S. platensis* diet groups, and that the Spirulina diet devoid of additional vitamins and minerals supported pregnancy performance equivalent to or better than the other diets in the study. They concluded that Spirulina appears to be a good dietary supplement during pregnancy.

In 1998 Kapoor and Mehta reported on the hematological parameters for pregnant females on the above five diets (Kapoor et al. 1998). The authors stated that higher iron storage and hemoglobin values were observed for females on *S. platensis* diets, regardless of the mineral and vitamin supplements. The researchers also noted that a decrease in iron storage and serum iron on day 20 of the study were pronounced in *S. platensis* fed rats, but could be attributed to the greater iron demand from larger litter sizes associated with the *S. platensis* fed rats.

Combined, these results suggest that *S. platensis* diets enhance pregnancy by increasing the weight gain during pregnancy, and that *S. platensis* diets increase litter sizes and promote healthy iron levels during pregnancy.

## **Safety assessment of other *Spirulina* species**

Various commercial *Spirulina* manufacturers generally refer to products simply as “*Spirulina*”, without specifying any particular strain. However, the most common commercially available strains are *S. platensis* and *S. maxima* (Khan et al. 2005). Although *S. platensis* is the subject of this particular GRAS notification, because *S. maxima* is a closely related strain and both are commonly used as a food source, the evaluation of *S. maxima* safety studies, as well as *Spirulina* safety studies that do not specify the species adds corroborative support to the safety evaluation of *S. platensis*.

A comprehensive *Spirulina* animal study sponsored by the United Nations Industrial Development Organization (UNIDO) investigated the sub-chronic and chronic toxicity of *Spirulina*, along with its effects on reproduction and lactation, as well as the teratogenic and mutagenic potential of the algae (Chamorro-Cevallos 1980). While the species of *Spirulina* is not reported in these studies, it is assumed to be *S. maxima* due to the manufacturer of the *Spirulina* used in the study (Sosa Texcoco Company—this company was the first large-scale manufacturer of *Spirulina maxima*, and the company is longer in business). A brief summary of this study and other literature-reported accounts that are not specific to *S. plantensis* are discussed below.

### **Sub-Chronic Oral Toxicity Studies**

In a 13-week sub-chronic study sponsored by UNIDO, Wistar rats fed a diet of 10, 20, and 30% *Spirulina*, as opposed to a soy-based diet, did not have any abnormalities with respect to weight, behavior, appearance, food consumption, hematologic or urologic parameters, GOT, GPT, alkaline phosphatase, macroscopic, histopathological or organ changes. The single statistically significant change observed in this study was the increase in relative weight of the seminal vesicles in male rats treated with 20 and 30% *Spirulina*. However, the authors concluded that this was not of toxicological interest as there were no pathological findings during histopathological examination.

In 1998 a 13-week sub-chronic study by Salazar and co-workers on the safety of *S. maxima* in CF1 mice, reported that upon organ examination at termination of the study, increased seminal vesicle weights were observed for male mice receiving *S. maxima* supplemented diets (Salazar et al. 1998). However, all other organs weights were similar to those of the control animals. Furthermore, macroscopic organ examinations did not show any organ abnormalities as a result of *S. maxima* treatment. Based on all the studied parameters (including food and water consumption, growth, behavior, survival, weight, organ and tissue examination, hematology, clinical chemistry and post-mortem

examinations) the authors concluded, "high feeding levels did not produce adverse effects in mice."

It is further necessary to mention that a comprehensive safety evaluation, published in Spirulina in Human Nutrition and Health, involving multiple Spirulina toxicological studies dating back to 1980 collectively found that Spirulina did not result in any treatment-related adverse effects in sub-chronic studies in rats and mice (receiving 10–60% Spirulina supplemented diets) (Gershwin and Belay 2008). The Spirulina diets had no effect on behavior, food and water intake, growth, survival, and organ weights. Terminal values in hematology and clinical chemistry did not reveal differences between treated and control groups. Post-mortem examination revealed no differences in gross or microscopic findings

### **Chronic Oral Toxicity Studies**

An 84-week chronic toxicity study, sponsored by UNIDO, investigated the effects of long-term 10%, 20%, and 30% Spirulina supplemented diet on hematological parameters, kidney function, serum chemistry parameters, and the weight and histopathology of certain organs in Wistar rats. Chamorro-Cevallos concluded that Spirulina did not produce any toxic effects in any of the parameters studied including tissue lesions and tumor incidence (Chamorro-Cevallos 1980).

Interestingly, in this long-term 84-week treatment period, the authors of the UNIDO-sponsored study did not observe any seminal vesicle increase (or decrease) for male rats receiving 0, 10%, 20% or 30% Spirulina supplemented diets. This result suggests that if there is indeed an effect on seminal vesicle weight change, then this observation/effect is not a consistent deleterious effect. Furthermore, histopathology examination did not report any tissue or organ abnormalities as a result of long-term exposure to Spirulina.

### **Reproductive and Developmental Toxicity Study**

To detect any effects of feeding Spirulina to successive generations, the UNIDO-sponsored multigenerational study on reproduction and lactation of Wistar rats was spread over a two-year period (three generations in approximately two years) and was completed in the last generation by a conventional sub-chronic toxicity study (Chamorro-Cevallos 1980). The results of the 13-week sub-chronic study performed on the third generation groups found significant differences in the urine specific gravity values in the male 20% Spirulina group, and in the female control group. Significant differences were also noted in the weights of the hearts, kidneys, and seminal vesicles of the males in the treatment groups and in the weights of the lungs and spleens of some females in the treatment groups. The differences were not dose-dependent and were not accompanied by pathological differences that could be attributed to Spirulina toxicity. Macroscopic examination revealed hydronephrosis in all groups, but no histopathologically examined lesions were attributed to Spirulina.

In other reproductive toxicity studies undertaken since Chamorro's and mentioned in his recent review, published in the book Spirulina in Human Nutrition and Health (Chapter 2), no developmental abnormalities have been

observed at any time between zygote formation and postnatal maturation in Spirulina-fed rats, mice or hamsters (Gershwin and Belay 2008). It has been reported that *S. maxima* treatment was not associated with any adverse effect on any measure of reproductive performance, including male and female fertility and duration of gestation (Salazar et al. 1996). All accounts on the reproductive performance of animals (mice and rats) receiving Spirulina supplemented diets reported no deleterious effects on reproduction or fertility as a result of Spirulina.

The objective of the UNIDO-sponsored teratogenicity studies was to detect any Spirulina-related embryonic resorptions or fetal malformations. Wistar rats, CD-1 mice, and Dorado hamsters born to mothers fed 10, 20, and 30% Spirulina diets were used. The Spirulina was fed to the treatment groups over three different periods during gestation. After the pregnant animals were weighed and sacrificed, fetuses of the sacrificed animals were counted, weighed, and examined for internal (visceral and skeletal) or external malformations; uterine wall implantations were counted to determine embryonic resorptions. From the data, a mean teratogenic index was calculated using the average percentages of control and treated animals affected. Some isolated cases of statistical significance occurred in the mean weight of the fetus, the number of implantations per fertile female, and the number of fetuses per pregnant female, but they did not show any relationship to the level of Spirulina fed to the animals. Chamorro's overall conclusion was that Spirulina does not cause gestational changes indicated by malformations, anomalies, or resorptions (Gershwin and Belay 2008).

Teratogenicity was also examined in Salazar's study (Salazar et al. 1996), in which *A. maxima* was administered in the diet of Wistar rats at levels of 10, 20 and 30%. Spirulina-fed rats were mated and a portion of the pregnant rats was allowed to give birth. Development of the pups was monitored for viability, weight, and attainment of developmental markers. A portion of the pups was reared to maturity and reproductive performance was assessed. None of the measures of reproductive performance, including fertility and gestation duration, were associated with any negative effects. Nor were there increases in the number of abnormal pups or adverse effects on developmental markers or reproductive performance in the F1 generation.

It is noteworthy that seminal vesicle weight increases were only observed in the short-term (13-week) toxicological studies, involving young animals, and the observation was not detected in the long-term UNIDO-sponsored chronic study, or in any of the studies that utilized *S. platensis* as the test article. Furthermore, upon examination no histopathological abnormalities were observed in the animals that exhibited seminal vesicle increases. Additionally reproductive and developmental abnormalities were not observed in the three-generation toxicity study. Therefore, it is concluded that the increased seminal vesicle weight increase seen in some studies is not of toxicological significance.

## **Additional Scientific Studies**

While Spirulina has been consumed for hundreds of years, a number of human clinical trials performed in recent years further support safety of oral administration of Spirulina species by, for example, confirming the absence of adverse effects. Some relevant human studies are summarized below.

- A randomized, double-blind, placebo-controlled cross-over study of Spirulina (species not indicated) in overweight adults was conducted by the Institut für Chemische Pflanzenphysiologie der Universität in Tübingen, Germany. The study consisted of 15 overweight patients with a mean age of 35.8 years and a mean BMI of  $30.5 \pm 5.2$ . Intake of 2.8 g Spirulina three times per day (8.4 grams total per day) for 4 weeks was associated with no adverse effects on blood pressure, heart rate, complete blood count, clinical chemistry panel, kidney function, enzyme activities, or physical symptoms (Becker et al. 1986).
- To evaluate the effects of *S. maxima*, sixteen males and 20 females, all healthy adults, orally consumed 4.5 grams Spirulina per day (3 tablets of 0.5 grams every 8 hours) for six weeks. At the beginning of the study and every week, fasting blood samples were taken and glucose, triacylglyceride, total cholesterol, HDL-cholesterol, and AST levels were determined to assess the potential hepatic effects of treatment. No changes were observed in AST and glucose values throughout the experimental period, and a hypolipidemic effect was seen with regard to the other markers studied. No adverse effects were reported and the study authors reported that safety of oral administration was demonstrated (Torres-Duran et al. 2007).
- Thirty men with cardiovascular disease risk factors were fed Spirulina in addition to their normal diet. Group A consumed 4.2 grams daily for eight weeks. Group B consumed Spirulina for four weeks and were observed for another four weeks. Fasting blood samples were collected and white blood cells, glutamate oxaloacetate transaminase, glutamic-pyruvate transaminase, lactate dehydrogenase, gamma-glutamyl transpeptidase, alkaline phosphatase, uric acid, BUN, and creatine were measured at weeks 0, 2, 4, 6, and 8. No significant changes occurred in any of these parameters. No adverse effects were reported by any of the subjects and no problems were found on clinical examination (Nakaya et al. 1988).
- A search of clinicaltrials.gov outlines studies intended to evaluate oral consumption of Spirulina at levels of 5 to 19 grams daily in various populations, suggesting that these levels are considered safe for use in those populations by Institutional Review Boards (trial numbers NCT00680277, NCT01141777, NCT01084382, NCT01195077).

## **Immunological-Specific Studies**

Immunomodulatory effects have been reported for Spirulina and/or extracts/components isolated from Spirulina in animal studies (Khan et al. 2005). However, the effects shown thus far in humans are small. In sub-populations in which it may be desirable to avoid immunomodulation (such as organ transplant

recipients that need to avoid interleukin (especially IL-2) stimulation), the effects at the levels of intended use stated in this GRAS notification should not be of concern. Relevant placebo-controlled studies are detailed below.

- In a study by Park and colleagues, seventy-eight subjects, aged 60–87 years, were given either 8 grams per day of Spirulina (obtained from Earth Spirulina Group, ES Co., Korea) or placebo for four months in a randomized double blind study (Park et al. 2008). The species of Spirulina was not specified. This study investigated the “antioxidant capacity, immunomodulatory and lipid-lowering effects” of Spirulina in elderly Korean males and females.

The participants consumed 8 grams (as 40 x 200 mg pills) each day of Spirulina or placebo, and blood samples at the end of the treatment period were compared to baseline blood samples. The plasma immunological parameters that were assessed using enzyme-linked immunosorbent assay (ELISA) techniques included IL-2, IL-6, TNF- $\alpha$  and MCP-1.

An increase in plasma IL-2 levels over baseline in males receiving Spirulina pills was reported. However, the baseline value for this treatment group was significantly lower than the baseline value for the placebo group (9.43 pg/mL and 13.2 pg/mL at baseline, respectively). After the 4-month treatment period there was no statistically significant difference between the IL-2 values for males in the Spirulina and placebo groups (13.6 pg/mL and 13.0 pg/mL, respectively).

In female subjects, both treatment and placebo baseline IL-2 values (9.39 pg/mL and 10.9 pg/mL, respectively) were lower than the final values (13.8 pg/mL and 13.3 pg/mL, respectively), and the rise in both groups reached statistical significance as compared to baseline ( $p < 0.05$ ). As was true in the male subjects, there was not a statistically significant difference between the treatment and placebo groups' final IL-2 values in female subjects.

Males in the Spirulina group also had a significant decrease in IL-6 as compared to baseline (2.64 pg/mL and 1.94 pg/mL), but again, the final value was not significantly different than that of the placebo group. Treated females had an increased IL-6 level as compared to baseline (1.02 pg/mL and 1.80 pg/mL) (which may have been statistically significant as indicated by an asterisk in the data table in the publication, however there is no mention of its significance in the body of the paper). Again there was no statistically significant difference between the treated and placebo IL-6 levels after four months of treatment. No treatment-related significant changes in TNF- $\alpha$ , MCP-1 and C3 (pro-inflammatory cytokines) levels were observed.

In conclusion, consumption of 8 grams per day of Spirulina may have led to mild immunomodulation of IL-2 and IL-6 in this population of elderly Korean subjects. However this is not entirely clear, as the final values for these cytokines remained within those considered “within the normal range” (for example, Mayo Medical Laboratories reports that normal IL-2 levels (ELISA assays) are  $< 35$  pg/mL, and normal IL-6 levels are  $< 17.4$  pg/mL. Published literature reflects similar values as normal (Tuncer et al. 2003; Ceyhan et al. 2004; Takahashi et al. 2010)). Additionally, the final values did not differ significantly from those of the placebo group. The authors did not

report any adverse events, and suggest that such slight immunomodulation could be of benefit in this population.

While this potentially mild immunostimulation after exposure to Spirulina at 8 grams per day should not be of concern for certain sensitive populations (such as transplant recipients, as mentioned previously), it is unclear how higher exposure levels (>8 g) would affect the immune system. The estimated exposure of Parry's Certified Organic Spirulina is at levels below the 8 grams per day that was utilized in this study. Future studies may elucidate the effects of higher doses of Spirulina on specific immune markers such as IL-2.

- In contrast to the previous study, Mao and colleagues found that a lower dose of a Spirulina-based dietary supplement (Earthrise Nutritionals, Inc.; Irvine, CA) did not cause significant changes in IL-2 (or IFN- $\gamma$ ) when given to subjects with allergies for 12 weeks (Mao et al. 2005).

The study was a randomized, double blind cross-over study with 36 subjects. The subjects were divided into three groups, and received placebo, 1 gram or 2 grams of Spirulina per day. Blood was collected at week zero (prior to treatment) and again at week 12. Cytokine measurements were achieved by stimulating peripheral blood mononuclear cells (PBMC) with the mitogen phytohemagglutinin (PHA), and measurements were done using ELISA assays. While significant changes were not seen in IL-2 or IFN- $\gamma$ , administration of two grams of Spirulina per day (the higher dose) did result in a statistically significant decrease in the production of IL-4 levels as compared to baseline ( $21.9 \pm 3.2$  and  $14.9 \pm 3.0$  pg/mL, respectively). It should be noted that according to the authors, the average baseline value for IL-4 from PHA-stimulated cells of the allergic patients in this study was nearly 70% higher than that of healthy individuals.

## Spirulina Consumption

### History of Consumption

According to numerous historical records, Spirulina has been a component of the everyday diet of certain human populations for hundreds of years. For instance, it is generally agreed that the blue-green algae gathered from Lake Texcoco by the ancient Aztecs, made into dried cakes called *tecuítlatl* and regularly consumed in the diet, was indeed *Arthrospira* (Johnston 1970) (Deng et al. 2010). Bernal Díaz del Castillo first described this food during the Spanish conquests led by Cortes. While exact quantities of *tecuítlatl* consumed are unrecorded, daily consumption appears to have been the norm. A similar food, called *dihé*, was prepared from Spirulina gathered by the Kanembu tribes from alkaline lakes near Lake Chad in central Africa. First described in 1940 by the French phycologist Dangeard, *dihé*, composed almost exclusively from *A. platensis*, is used to make soups and sauces to accompany millet, and is still regularly consumed by the local populations near Lake Chad (Ciferri 1983; Habib et al. 2008). Based on surveys in Chad, frequency of consumption varies from one to six meals out of ten, and between nine and thirteen grams of Spirulina are consumed per person during a meal (Delpeuch et al. 1975). Detailed accounts of

the historical human consumption of other species of blue-green algae, including *Nostoc flagelliforme*, *Phyllocladus sacrum*, and *Prasiola japonica*, also appear in the literature (Johnston 1970).

Commercial Spirulina production began in the 1970's. Today, *A. platensis* is one of the most commercialized micro algae, being cultivated for mass production for use in human food, animal feed, and colorimetric industry (Kim et al. 2007). It has been sold both in the United States and around the world since the late 1970s as a food product and dietary supplement. More than 3,000 tons of *A. platensis* are produced annually worldwide, and the majority is used for health food products and animal feed additives (Eriksen 2008).

### **Previous Sales and Reported Adverse Events**

Parry Nutraceuticals' Organic Spirulina Powder is currently sold in the United States as a dietary supplement, as well as both a dietary supplement and a food ingredient in other parts of the world. Since the year 1996, Parry Nutraceuticals has sold 1600 metric tons of Spirulina for use as dietary supplements, and has received no reports of serious adverse events.

To the best of our knowledge the FDA has not issued any letters regarding concern for safety to companies that market products containing *A. platensis* or any other *Arthrospira* species.

### **Current Regulatory and International Status**

Spirulina has regulatory status as a food and dietary supplement in the United States, and is recognized by various organizations internationally as an important food source. This information is corroborative to the determination that Parry Nutraceutical's Certified Organic Spirulina is GRAS. Pertinent information in this regard is outlined in the section below:

- An *A. platensis* powder was the subject of a previous GRAS determination, submitted on behalf of Cyanotech Corporation and Earthrise Nutritionals, Inc. (GRAS notification #127). On the basis of scientific procedures, these two companies determined their spray-dried Spirulina powder is GRAS when used as an ingredient in foods such as bars, powdered nutritional drink mixes, popcorn, and as a condiment in salads and pasta, at levels ranging from 0.5 to 3 grams per serving size. FDA responded on October 6, 2003, having no questions regarding the notifiers' conclusion that Spirulina is GRAS under the intended conditions of use. Parry Nutraceuticals' Certified Organic Spirulina is bioidentical to the subject of the previous notification, and follows the same raceway pond method of manufacturing.
- An ingredient containing *A. platensis* grown in a selenium-rich medium, in combination with vitamin E and superoxide dismutase microgranules, was the subject of a New Dietary Ingredient (NDI) notification by the company VitaBioTech LLC (NDI notification #648). The daily serving size of the combined ingredient was stated as 400 mg per day. FDA filed the notification on June 18<sup>th</sup>, 2010, with no further questions for the notifier.

- The Convention for the Use of Food Micro-Algae, Intergovernmental Institutional Spirulina Program (CISRI-ISP/ IIMSAM) works to promote the use of Spirulina against severe malnutrition. It has been established through two international agreements that are recognized in the UN Treaty Series. IIMSAM is accredited as a Permanent Observer Mission with the United Nations Economic and Social Council.
- Spirulina was recommended as one of the primary foods during long-term space missions by both the National Aeronautics and Space Administration (NASA) and the European Space Agency (ESA) (Deng and Chow 2010) (Habib and Parvin 2008).
- Clinical studies in India and Africa have shown that, when Spirulina is used as a food complement, there is a significant response in the improved nutritional status of undernourished children (Simpore et al. 2006). The European Commission's Humanitarian Aid department (ECHO) funds supplemental feeding programs for about 65,000 Sri Lankan war refugees. The project funds *A. platensis* farming as the main food source of a low budget, high nutrition diet.
- Antenna Technology is a Swiss-based organization composed of scientists and researchers working on issues of malnutrition by introducing Spirulina as a tool to fight childhood malnutrition. Antenna's publication, *The Nutritional Aspects of Spirulina* cites a study at Hôpital Bichat, France, of malnourished children and adults who were given doses of 80–90 grams Spirulina per day. Absorption of Spirulina proteins was found to be good, and despite these very large doses, no noteworthy increase in blood uric acid was demonstrated. The original publication verifying this reference could not be obtained (Falquet 1996).
- Shri AMM Murugappa Chettiar Research Centre (MCRC), Chennai, India is a non-Governmental Voluntary Research Organization established in 1977. The MCRC trains rural people especially women to grow Spirulina for nutrition and income generation. They conducted an 18-month trial with 5,000 pre-school children in India and showed a decrease in Bitot's spots (a symptom of Vitamin A deficiency) from 80% to 10% after consumption of 1 g Spirulina per day for at least 150 days.
- The Food and Agriculture Organization of the United Nations considers Spirulina to have significantly high macro- and micronutrient content, and authors representing the organization discuss Spirulina's important use as human food in its 2008 document entitled "A Review on Culture, Production and Use of Spirulina as a Food for Humans and Feeds for Domestic Animals and Fish" (Habib and Parvin 2008).
- The USDA National Nutrient Database for Standard Reference lists the nutrient data for dried Spirulina, suggesting that Spirulina is considered a food. Nutrient values are given for 7 g and 112 g of dried Spirulina. This database is the major source of food composition data in the United States, and provides the foundation for most food composition databases in the public and private sectors.

## Information that may appear to be Inconsistent with GRAS Determination of Spirulina

Spirulina has a long history of consumption by humans as a food source, and numerous animal and human studies have demonstrated no safety concerns with regard to this ingredient. There have been rare adverse incidences that have coincided with consumption of Spirulina supplements, although Spirulina causation has not been proven. A single case study was reported concerning a 28-year-old man who developed rhabdomyolysis after taking Spirulina for one month. He reported that he was not taking other supplements or medications and had no other risk factors (Mazokopakis et al. 2008). In contrast, a small clinical study showed potential preventive effects of exercise-induced skeletal damage (Lu et al. 2006). Another single case study was published of a 52-year-old Japanese man who showed signs of hepatotoxicity after taking Spirulina, although he was also taking three other medications. The signs resolved after discontinuation of the Spirulina and all medications (Iwasa et al. 2002). It is impossible to know if the hepatotoxicity was related to Spirulina consumption in this case, and other such cases have not been reported to the best of our knowledge.

Lastly, in 2010 Petrus and co-workers reported on a single case study in which the consumption of Spirulina led to anaphylaxis in a 14-year-old adolescent (Petrus et al. 2010). No dose level was provided in the report. The boy suffered urticaria, labial edema and asthma six hours after consumption of five Spirulina tablets. The authors identified the allergen to be the  $\beta$ -chain of the protein C-phycoyanin.

In comment to this single report it is necessary to also refer to a 1997 study by Yang and co-workers on the anaphylactic inhibition action of *S. platensis* (Yang et al. 1997). Researchers investigated the effects of *S. platensis* on rats subjected to anaphylactic reactions. In the first experiment the rats were pretreated with *S. platensis* saline solutions (0.005–1.0 mg/g/bw), followed by compound 48/80 (8  $\mu$ g/g/bw), which induces mast cell histamine secretion. The authors found that 0.5 and 1.0 mg/g/bw *S. platensis* were effective at inhibiting systemic anaphylaxis with 0% mortality. This compared to 100% mortality for the 0 and 0.005 mg/g/bw *S. platensis* groups, and 80%, 60% and 30% mortality for the 0.01, 0.05 and 0.1 mg/g/bw groups, respectively. Serum analysis further showed a 62.7% inhibition rate for animals in the 1.0 mg/g body weigh *S. platensis* group (n=7) compared to 0% inhibition for animals receiving the vehicle (saline).

In a second experiment the authors tested the inhibition ability of *S. platensis* on IgE-mediated anaphylaxis. During this experiment rats injected with 100  $\mu$ g of anti-DNP IgE, received 0, 0.1 and 0.5 mg/g/bw *S. platensis* one hour prior to injection with 1 mg DNP-HSA and Evans dye. Upon analysis rats in the 0, 0.5 and 1.0 mg/g/bw group had 0%, 40.8% and 68.7% inhibition rates, respectively. Combined, the results of these experiments showed *S. platensis* acts as an effective inhibitor of anaphylactic reactions.

Hence, while there is a single case study showing an allergic reaction to *S. platensis*, Spirulina has been consumed widely in many countries for many years, and to our best knowledge no other Spirulina-related allergic reactions have been reported. Therefore the occurrence of allergenic reactions to Spirulina appears to be extremely rare. Furthermore, in the animal study mentioned above, *S. platensis* was shown to have potent anti-anaphylactic properties.

## **Intended Use**

### **Categories of Food**

For the purpose of this GRAS self-affirmation, Parry Nutraceutical's Certified Organic Spirulina, manufactured in accordance with Good Manufacturing Practice (GMP) as specified in 21 CFR 110, is intended to be used as an ingredient in the categories of food discussed below.

Parry Nutraceutical's Certified Organic Spirulina is not intended for use in infant formula; or in meat, egg or catfish products, which would require additional review by USDA. Parry Nutraceutical's Certified Organic Spirulina is not intended for use as a color additive as per 21 CFR 70.3 (f), although like cherries, green or red peppers, chocolate and orange juice, it contributes its own natural color when mixed with other foods. As per 21 CFR 70.3 (g), it will be used in a way that any color imparted is clearly unimportant insofar as the appearance, value, marketability or consumer acceptability is concerned, and hence is exempt from FDA premarket approval requirements for color additives.

Parry Nutraceutical's Certified Organic Spirulina may be added to the following category of foods as defined in 21 CFR §170.3(n):

- (3) Beverages and beverage bases, nonalcoholic, including only special or spiced teas, soft drinks, coffee substitutes, and fruit and vegetable flavored gelatin drinks.
- (4) Breakfast cereals, including ready-to-eat and instant and regular hot cereals.
- (16) Fresh fruits and fruit juices, including only raw fruits, citrus, melons, and berries, and home-prepared "ades" and punches made therefrom.
- (20) Frozen dairy desserts and mixes, including ice cream, ice milks, sherbets, and other frozen dairy desserts and specialties.
- (23) Grain products and pastas, including macaroni and noodle products, rice dishes, and frozen multicourse meals, without meat or vegetables.
- (31) Milk products, including flavored milks and milk drinks, dry milks, toppings, snack dips, spreads, weight control milk beverages, and other milk origin products.

(33) Plant protein products, including the National Academy of Sciences/National Research Council "reconstituted vegetable protein" category, and meat, poultry, and fish substitutes, analogs, and extender products made from plant proteins.

(35) Processed fruits and fruit juices, including all commercially processed fruits, citrus, berries, and mixtures; salads, juices and juice punches, concentrates, dilutions, "ades", and drink substitutes made therefrom.

(36) Processed vegetables and vegetable juices, including all commercially processed vegetables, vegetable dishes, frozen multicourse vegetable meals, and vegetable juices and blends.

(37) Snack foods, including chips, pretzels, and other novelty snacks.

(38) Soft candy, including candy bars, chocolates, fudge, mints, and other chewy or nougat candies.

(40) Soups and soup mixes, including commercially prepared meat, fish, poultry, vegetable, and combination soups and soup mixes. This excludes foods that fall under USDA jurisdiction, such as;

- Catfish (USDA proposed rule 76FR10434)
- Soups that include more than "relatively small portions" meat and poultry within the products. Relatively small portions are as defined by 9 CFR 381.15 and the 2005 USDA Food Standards and Labeling Policy Book: 3 percent or less raw meat; less than 2 percent cooked meat or other portions of the carcass; or 30 percent or less fat, tallow or meat extract, alone or in combination. In the case of poultry, less than 2 percent cooked poultry meat; less than 10 percent cooked poultry skins, giblets or fat, separately; or less than 10 percent cooked poultry skins, giblets, fat and poultry meat (limited to less than 2 percent) in any combination.
- Soups that include more than "relatively small proportions" of egg (as defined in 9 CFR 590.5 (h), p. 660 under subtitle "Egg product").

### **Estimated Daily Intake (EDI)—Exposure**

Organizations around the world generally encourage consumption of Spirulina rather than limit it (Habib and Parvin 2008). In order to calculate an estimated daily intake (estimated exposure) for Certified Organic Spirulina, addition levels of the ingredient per serving as well as number of servings to be consumed daily were considered. According to a USDA Nutrition Insights article (a publication of the USDA Center for Nutrition Policy and Promotion), Insight 20 October 2000 (Basiotis et al. 2000), males aged 51 or greater consume the greatest number of servings of total food per day. This group consumes an average of 18.2 servings of food per day, totaled from the following food groups: grains, fruits, vegetables, milk, meat and other (fats, oils, sweets). According to the same publication, females aged 19–24 consume the smallest number of total daily servings of food, at 12.5 total servings per day. Because Certified Organic

Spirulina is intended for use in foods categories that span all of these food groups, it is reasonable to consider this data when calculating the estimated daily intake of Spirulina.

Certified Organic Spirulina will be added to foods in the categories listed in the previous section, at a level of 0.5–3 grams per serving. If 10% of a person's daily food intake contained Certified Organic Spirulina, then the highest food consumers (based on the previously mentioned USDA article) would eat 1.82 servings of Spirulina per day. If we round this to 2.0 servings, then a high-end consumer is estimated to consume up to 6 grams per day of Certified Organic Spirulina. On the other hand, using this same methodology, a low-end food consumer is estimated to consume 1.25 servings of Spirulina per day, with a total exposure of approximately 3.75 grams per day. This is likely an over estimate, because, as discussed below, individuals tend to seek out Spirulina and Spirulina products, so a low-end user would likely consume very little of this ingredient.

Spirulina is already present in the marketplace in many of the categories that are included in this notification, such as fruit and vegetable juices and cereals. Recipes are also readily available on the internet for addition of Spirulina to foods such as soups, or smoothies that contain milk. Spirulina is sold in a powdered form as a dietary supplement, allowing for the addition of it to smoothies, etc. by consumers. This suggests that individuals who are interested in adding Spirulina to their diets are seeking it out, and adding it to their diets intentionally, via, for example, home-cooked foods. The availability of individual products in the marketplace that contain Spirulina would likely replace consumption of related home-cooked products for such individuals. Similarly, one brand of Spirulina in a product would likely replace another in a particular category of food purchased by a consumer. Therefore, consumption of Certified Organic Spirulina is expected to replace the consumption of other brands of Spirulina, and is not expected to add significantly to the exposure of individuals who are already consuming a Spirulina ingredient.

## **General Recognition**

The scientific studies that provide the basis of this GRAS determination by scientific procedures, and information related to the historical consumption of Spirulina as well as additional scientific studies which corroborate the scientific safety data, are published and available in the public domain. The reference section of this notification contains the citations for these published studies. This published data, along with government positions that promote the use of Spirulina as a food, as well as the broad availability of this ingredient in the marketplace, provide ample evidence of consensus among qualified experts that there is reasonable certainty that consumption of Certified Organic Spirulina is not harmful. The general availability of this information satisfies the common knowledge component of this GRAS notification.

## **Basis for the GRAS Determination**

Based on an independent and collective critical evaluation of the data and information described above, the Expert Panel, qualified by scientific training and experience to evaluate the safety of substances added to food, concluded that Parry Nutraceutical's Certified Organic Spirulina, when produced according to Good Manufacturing Practice and meeting the specifications presented in this notification, is generally recognized as safe for its intended use based on scientific procedures, and is hence exempt from the requirement of premarket approval. The totality of evidence for the safety of Certified Organic Spirulina is made up of both pivotal and corroborative data. Pivotal data for this determination includes the various *in vitro*, short and long-term toxicological studies in the scientific public domain that have been performed on *S. plantensis*, which are summarized and discussed in the so-named section of this notification, and provide no concerns with regard to the safety of consumption of this species. Pivotal information also relates to the high quality control standards for this ingredient. The previous FDA GRAS notification No. 127 for Spirulina (*S. platensis*), which was filed by FDA without question, should also be noted as important information that helped form the basis of the determination. This data was corroborated by safety studies performed on other species of Spirulina, (including animal and human studies) as well as a long history of safe consumption of Spirulina, and the opinions and consumption recommendations of various organizations mentioned in this notification. A plethora of publications in the public domain, including human and animal studies, demonstrate that there is common knowledge and consensus among qualified experts that Spirulina is safe for its intended use.

## References

- Basiotis P, Lino M, et al. "Consumption of Food Group Servings: People's Perceptions vs. Reality." *Nutrition Insights* 20, 1-2. 2000.
- Becker EW, Jakover B, et al. Clinical and biochemical evaluations of the alga *Spirulina* with regard to its application in the treatment of obesity: a double blind cross-over study. *Nutr Rep Int.* 1986; 33: 565-574.
- Ceyhan BB, Enc FY, et al. IL-2 and IL-10 levels in induced sputum and serum samples of asthmatics. *J Investig Allergol Clin Immunol.* 2004; 14: 80-85.
- Chamorro-Cevallos G. Toxicological Studies on *Spirulina* Alga Sosa Texcoco S.A. Pilot Plant for the Production of Protein from *Spirulina* Alga, United Nations Industrial Development Organization; 1980.
- Ciferri O. *Spirulina*, the edible microorganism. *Microbiol Rev.* 1983; 47: 551-578.
- Delpeuch F, Joseph A, et al. [Consumption and nutritional contribution of the blue algae (*Oscillatoria platensis*) among some populations of Kanem (Tchad)]. *Ann Nutr Aliment.* 1975; 29: 497-516.
- Deng R and Chow TJ. Hypolipidemic, antioxidant, and antiinflammatory activities of microalgae *Spirulina*. *Cardiovasc Ther.* 2010; 28: e33-45.
- Desikachary IV and Bai NJ. Taxonomic studies in *Spirulina* II. The identification of *Arthrospira* ("Spirulina") strains and natural samples of different geographical origins. *Algological Studies.* 1996; 83: 163-178.
- Endo H, Hosoya H, et al. Isolation of 10-Hydroxypheophorbide a as a Photosensitizing Pigment from Alcohol-treated *Chlorella* Cells. *Agricultural and Biological Chemistry.* 1982; 46: 2183-2193.
- Eriksen NT. Production of phycocyanin--a pigment with applications in biology, biotechnology, foods and medicine. *Appl Microbiol Biotechnol.* 2008; 80: 1-14.
- Falquet J. *Spiruline, Aspects nutritionnels.* *Publicaciones Antenna Technology.* 1996.
- Garrity GM. *Bergey's Manual of Systematic Bacteriology:* Springer; 2005
- Gershwin ME and Belay A, Eds. *Spirulina in Human Nutrition and Health.* Boca Raton: CRC Press; 2008.
- Habib M and Parvin M. A review on culture, production and use of spirulina as food for humans and feeds for domestic animals and fish. FAO Fisheries and Aquaculture Circular No. 1034. Rome, Food and Agriculture Organization of the United Nations (FAO); 2008: 1-41.

- Hutadilok-Towatana N, Reanmongkol W, et al. A subchronic toxicity study of *Spirulina platensis*. *Food Sci Technol Res*. 2008; 14: 351-358.
- Iwasa M, Yamamoto M, et al. *Spirulina*-associated hepatotoxicity. *Am J Gastroenterol*. 2002; 97: 3212-3213.
- Johnston HW. The biological and economic importance of algae, Part 3: Edible algae of fresh and brackish waters. *Tuatara*. 1970; 18: 19-35.
- Kapoor R and Mehta U. Effect of supplementation of blue green alga (*Spirulina*) on outcome of pregnancy in rats. *Plant Foods Hum Nutr*. 1993; 43: 29-35.
- Kapoor R and Mehta U. Supplementary effect of spirulina on hematological status of rats during pregnancy and lactation. *Plant Foods Hum Nutr*. 1998; 52: 315-324.
- Khan Z, Bhadouria P, et al. Nutritional and therapeutic potential of *Spirulina*. *Curr Pharm Biotechnol*. 2005; 6: 373-379.
- Kim CJ, Jung YH, et al. Factors indicating culture status during cultivation of *Spirulina* (*Arthrospira*) *platensis*. *J Microbiol*. 2007; 45: 122-127.
- Lu HK, Hsieh CC, et al. Preventive effects of *Spirulina platensis* on skeletal muscle damage under exercise-induced oxidative stress. *Eur J Appl Physiol*. 2006; 98: 220-226.
- Mao TK, Van de Water J, et al. Effects of a *Spirulina*-based dietary supplement on cytokine production from allergic rhinitis patients. *J Med Food*. 2005; 8: 27-30.
- Mazokopakis EE, Karefilakis CM, et al. Acute rhabdomyolysis caused by *Spirulina* (*Arthrospira platensis*). *Phytomedicine*. 2008; 15: 525-527.
- Nakaya N, Homma Y, et al. Cholesterol lowering effect of *Spirulina*. *Nutr. Rep. Int*. 1988; 37: 1329-1337.
- Park HJ, Lee YJ, et al. A randomized double-blind, placebo-controlled study to establish the effects of spirulina in elderly Koreans. *Ann Nutr Metab*. 2008; 52: 322-328.
- Petrus M, Culerrier R, et al. First case report of anaphylaxis to spirulin: identification of phycocyanin as responsible allergen. *Allergy*. 2010; 65: 924-925.
- Salazar M, Chamorro GA, et al. Effect of *Spirulina maxima* consumption on reproduction and peri- and postnatal development in rats. *Food Chem Toxicol*. 1996; 34: 353-359.
- Salazar M, Martinez E, et al. Subchronic toxicity study in mice fed *Spirulina maxima*. *J Ethnopharmacol*. 1998; 62: 235-241.

- Simpore J, Kabore F, et al. Nutrition rehabilitation of undernourished children utilizing Spiruline and Misola. *Nutr J.* 2006; 5: 3.
- Takahashi H, Tsuji H, et al. Serum cytokines and growth factor levels in Japanese patients with psoriasis. *Clin Exp Dermatol.* 2010; 35: 645-649.
- Tomaselli L, Palandri MR, et al. On the correct use of the Spirulina designation. *Algological Studies.* 1996; 83: 539-548.
- Torres-Duran PV, Ferreira-Hermosillo A, et al. Antihyperlipemic and antihypertensive effects of Spirulina maxima in an open sample of Mexican population: a preliminary report. *Lipids Health Dis.* 2007; 6: 33.
- Tuncer I, Ozbek H, et al. The serum levels of IL-1 $\beta$ , IL-6, IL-8 and TNF- $\alpha$  in nonalcoholic fatty liver. *Turk J Med Sci.* 2003; 33: 381-386.
- Vonshak A, Ed. Spirulina platensis (Arthrospira): Physiology, Cell-biology and Biotechnology. Bristol, PA: Taylor & Francis; 1997.
- Yang HN, Lee EH, et al. Spirulina platensis inhibits anaphylactic reaction. *Life Sci.* 1997; 61: 1237-1244.
- Yang Y, Park Y, et al. In vitro and in vivo safety assessment of edible blue-green algae, Nostoc commune var. sphaeroides Kutzing and Spirulina platensis. *Food Chem Toxicol.* 2011; 49: 1560-1564.

SUBMISSION END

000036