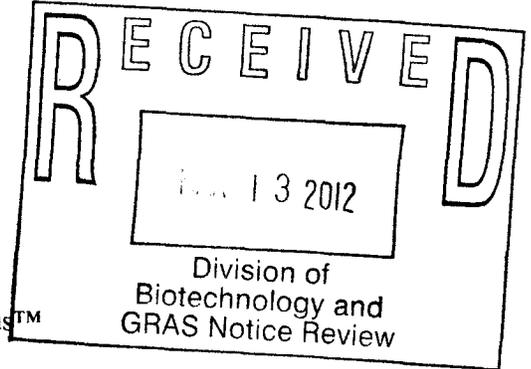


ORIGINAL SUBMISSION



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



www.desertlake.com

March 12, 2012

P.O. Box 489

ATTN: Dr. Antonia Mattia, PhD

Klamath Falls, Oregon 97601

Our Reference: GRAS Notification for CyaninPlus™

Tollfree 800-736-2379

Phone 541-885-6947

Dear Dr. Mattia,

Fax 541-885-6951

Desert Lake Technologies, LLC is submitting the attached GRAS notification to the FDA for CyaninPlus™, which is intended for use as an ingredient in food. CyaninPlus™ is a water extract of Spirulina, containing greater than 30% c-phycoerythrin. The GRAS determination has been made based on scientific procedures, using safety assessments of Spirulina and c-phycoerythrin, and is corroborated by a long history of safe consumption of Spirulina. The water extraction method of manufacturing does not chemically alter the ingredient and batch analyses show that the product is made consistently and meets all specifications. Therefore the intended use of CyaninPlus™ is exempt from the requirement of pre-market approval, consistent with section 201 (s) of the Federal Food, Drug and Cosmetic Act.

Please find enclosed three copies of the notification: *Notice to US Food and Drug Administration that the use of CyaninPlus™ is Generally Recognized as Safe*. Also enclosed are copies of all references cited in the notification. 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 our offices: Desert Lake Technologies, LLC, 3735 Washburn Way, Klamath Falls, Oregon 97603, USA, or will be sent to FDA upon request. Please do not hesitate to contact us with any questions.

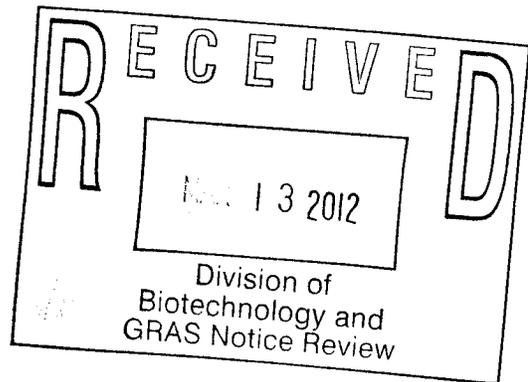
Yours sincerely,

Office

Howard Newman
Desert Lake Technologies, LLC
3735 Washburn Way
Klamath Falls, Oregon 97603
USA

3735 Washburn Way

Klamath Falls, OR 97603



**Notice to US Food and Drug Administration
that the use of CyaninPlus™ is Generally
Recognized as Safe**

Submitted by the Notifier:

Desert Lake Technologies, LLC
3735 Washburn Way
Klamath Falls, Oregon 97603, USA

March 8, 2012

Table of Contents

GRAS Exemption Claim.....	3
Name and Address of the Notifier	3
Common or Usual Name.....	3
Conditions of Use	3
Basis for GRAS Determination.....	3
Data/Information Availability Statement	3
Characterization.....	4
Manufacturing and Production	6
Company Overview	6
Manufacturing Overview.....	6
Good Manufacturing Practice.....	6
Specifications	7
Batch Analysis and Quality Management.....	7
Heavy Metal Analysis	8
Residual Solvent Analysis	8
Residual Pesticide Analysis.....	9
Microcystins.....	9
Shelf-life Stability	9
Self-limiting Levels of Use.....	9
Safety Assessment	10
Toxicology Studies	10
Acute Oral Toxicity.....	10
Sub-Chronic Oral Toxicity.....	11
Chronic Oral Toxicity	13
Reproductive/Developmental Toxicity	14
Human Studies	16
Immunological Studies	16
History of Consumption	18
Information that may appear to be Inconsistent with GRAS Determination	18
Current Regulatory Status.....	19
Regulation of Color Additives	20
Intended Use and Estimated Daily Intake (EDI)	20
General Recognition	22
Basis for the GRAS Determination	22
References.....	23

GRAS Exemption Claim

Desert Lake Technologies, LLC (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 CyaninPlus™ 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 CyaninPlus™ for its intended purpose is exempt from the requirement of pre-market approval.

(b) (6)

Howard Newman,
Desert Lake Technologies, LLC

3-8-2012

Date

Name and Address of the Notifier

Howard Newman
Desert Lake Technologies, LLC
3735 Washburn Way
Klamath Falls, Oregon 97603
USA

Common or Usual Name

CyaninPlus™ is a water extract from the cyanobacteria *Spirulina (Arthrospira platensis or Arthrospira maxima)*

Conditions of Use

CyaninPlus™ is intended for use as an ingredient in food, at levels of up to 250 mg per serving.

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 Desert Lake Technologies, LLC, 3735 Washburn Way, Klamath Falls, Oregon 97603, USA; or will be sent to FDA upon request.

Characterization

CyaninPlus™ is a water extract from the cyanobacteria *Spirulina* (*Arthrospira maxima* or *Arthrospira platensis*). These two species are commonly referred to in the literature by their traditional names of *Spirulina maxima* and *Spirulina platensis*. They are also the two species most commonly consumed as nutritional supplements, often referred to as simply *Spirulina* (Khan et al. 2005). The taxonomic classification of these organisms is as follows: kingdom Bacteria; phylum Cyanobacteria; order Oscillatoriales; family Phormidiaceae.

One of the earliest accounts of the use of *Spirulina* as a food source dates back to 1521 where the alga was harvested from Lake Texcoco in Mexico, and sold as a food (Ciferri 1983; Sanchez et al. 2003; Gershwin et al. 2008). The commercial production of *Spirulina* for human consumption dates back to the 1970's. One of the earliest large-scale *Spirulina* production operations, harvesting 2 tons (dry weight) per day, was also at Lake Texcoco in Mexico (Ciferri 1983). The nutritional value of *Spirulina* has been shown to include 60–70% protein, 0.3% vitamin B12 and β -carotene, 10–20% phycocyanin, 1–1.5% γ -linolenic acid, 1–2.5% sulfolipids and small quantities of iron (Tomaselli et al. 1996; Gershwin and Belay 2008) Desert Lake Technologies in-house data).

A major constituent (> 30%) of the CyaninPlus™ ingredient is the highly water-soluble phycobiliprotein c-phycocyanin (CAS registry number 11016-15-2). Phycobiliproteins are a family of heterodimeric proteins, containing homologous subunits with a globin-type core. The proteins contain chromophores (the part of the molecule that absorbs light and provides color), as well as a N-terminal extension involved in extensive oligomerization. Each phycobiliprotein contains a specific number of binding sites for chromophores, and has specific absorption and fluorescence emission wavelengths. The complex oligomerization process, which includes the formation of trimers, hexamers and decamers, forms extra-membraneous antenna complexes called phycobilisomes, which are involved in the harvest of light (Scheer et al. 2008). Amino acid variation of phycocyanins between species of cyanobacteria and red algae are very minor (Eriksen 2008).

In organisms, the main function of phycobiliproteins is to aid in the photosynthetic process (in conjunction with chlorophyll and carotenoids), whereby they assist in the light harvest at wavelengths where chlorophylls absorb poorly (Padyana et al. 2001; Scheer and Zhao 2008; Silveira et al. 2008). Phycobiliproteins in cyanobacteria are divided into four major classes: c-phycocyanin, phycoerythrin, phycoerythrocyanin and allophycocyanin. Spectroscopic analysis of phycocyanin content in CyaninPlus™ shows absorbance at 620 nm, and is specific to c-phycocyanin. In this regard, the terms c-phycocyanin and phycocyanin are considered synonymous terms in this notification when related to CyaninPlus™. Allophycocyanin appears spectroscopically as a small "shoulder", at 650 nm under the c-phycocyanin peak, and is not routinely quantified.

Phycocyanin is composed of two protein subunits—alpha and beta—which contain at least three covalently bound bilin chromophores via thioether linkages to specific cysteinyl residues (Romy et al. 1998; Bhat et al. 2000; Romy et al. 2003; Eriksen 2008). Phycocyanobilin is the chromophore of phycocyanin.

nitrogen conditions (El-Baky 2003). In-house testing by Desert Lake Technologies reveals that phycocyanin constitutes 10–20% of the Spirulina material from which CyaninPlus™ is manufactured, and composes a minimum of 30% of the final CyaninPlus™ product. The remainder of the product contains compounds from the parent microalgae that accompany phycocyanin during the aqueous extraction process.

Manufacturing and Production

Company Overview

Desert Lake Technologies, LLC is a vertically integrated harvester, processor and distributor of natural, fresh water products, mainly originating from Klamath Lake, Oregon. They provide raw materials for commercial and private label ingredients in the nutraceutical, dietary supplement, food, beverage, cosmetic, and pharmaceutical industries.

Dedicated to the discovery of the unique characteristics of microalgae, in 2003 Desert Lake Technologies and Merle West Center for Medical Research co-partnered with the creation of Lake Algae Research, LLC. Desert Lake Technologies continues to sponsor research through the laboratory of Dr. Gitte Jensen, NIS Labs, to study the distinctive attributes of microalgae.

Manufacturing Overview

CyaninPlus™ is manufactured using Spirulina (*Arthrospira platensis* or *maxima*) as the raw material. The feedstock is prescreened to assure the impurities are within relative limits and the phycocyanin levels are acceptable. The source of the Spirulina may vary as long as the final product meets Desert Lake Technologies' specifications, including a phycocyanin content of > 30%, while maintaining the specified limits of impurities.

The manufacturing process follows the following methodology: Spirulina powder is reconstituted with water (the only solvent used in the manufacturing process) and the aqueous portion is extracted using centrifugation. The aqueous material is then dried either by the DLT Hydro•Dri™ method or freeze-drying. The DLT Hydro•Dri™ method uses convection heat transfer through a food grade Mylar belt to remove free water.

The moisture content of the product is measured during manufacturing, and the drying equipment is adjusted accordingly. The product is then sized and or blended to customer request. Samples are taken for quality control testing purposes. The finished product is packaged to customer request using appropriate food grade material and then staged in a QC holding area where it awaits a Certificate of Analysis.

Good Manufacturing Practice

The manufacturing process for CyaninPlus™ is performed using current cGMP standards, as set to comply with the FDA's Code of Federal Regulations, 21 CFR

Part 111. With implementation of these regulations, all of Desert Lake Technologies' vendors, contract suppliers, contract laboratories, and employees are required to adhere to all requirements of Desert Lake Technologies' cGMP program.

Specifications

The product specifications for CyaninPlus™, including physical characteristics, microbiology, heavy metals, pesticides and toxins, along with the specification methods, are listed in **Table 1** below.

Table 1. CyaninPlus™ Specifications.

Physical Characteristics	Specification	Methods
Appearance	Ground powder (2–70 mesh)	Visual
Color	Brilliant Blue	Visual
Odor/Taste	Slight marine	Organoleptic
Moisture	2–7 %	Gravimetric
Density	0.2–0.7 g/mL	USP
C-phycocyanin	> 30 %	Spectrophotometric (620 nm absorbance)
Microbiology		
Total Plate Count	< 10,000 cfu/g	AOAC 990.12
Total Coliforms	< 10 cfu/g	AOAC 991.14
Mold and Yeast	< 1,000 cfu/g	AOAC 997.02
<i>E. coli</i>	Negative/g	AOAC 991.14
<i>S. aureus</i>	< 10 cfu/g	AOAC 2001.05
Salmonella	Negative/10 g	FDA BAM
Heavy Metals		
Arsenic (inorganic)	≤ 1 ppm	ICP/MS AOAC 993.14
Cadmium	≤ 1 ppm	ICP/MS AOAC 993.14
Lead	≤ 1 ppm	ICP/MS AOAC 993.14
Mercury	≤ 1 ppm	ICP/MS AOAC 993.14
Pesticides and Toxins		
Microcystin Toxin	None detected (limit of detection = 0.16 ppm)	ELISA
Organophosphorus Pesticides	None detected	EPA 8141B
Organochloride Pesticides	None detected	EPA 8081B
Pyrethroid Pesticides	None detected	EPA 8270D

Batch Analysis and Quality Management

CyaninPlus™ production consistency is tested in production lots. As shown in **Table 2** below, three batches examined were reasonably consistent and met the product specifications for physical/general composition, phycocyanin content, heavy metals, microbial analyses and the absence of microcystin and pesticides.

Table 2. CyaninPlus™ Batch Analyses

	Specification	Batch Numbers		
		DLTL-01 93-1	DLTL-01 93-2	DLTL-01 93-3
Physical Characteristics				
Appearance	Brilliant Blue	Brilliant Blue	Brilliant Blue	Brilliant Blue
Moisture	2-7 %	5.7 %	4.9 %	4.6 %
C-phycocyanin	> 30 %	42.2 %	46.6 %	43.8 %
Microbiology				
Aerobic Plate count	< 10,000 cfu/g	3700 cfu/g	4400 cfu/g	1100 cfu/g
Yeast and Mold	< 1,000 cfu/g	< 100 cfu/g	< 100 cfu/g	< 100 cfu/g
Coliforms	< 10 cfu/g	< 10 cfu/g	< 10 cfu/g	< 10 cfu/g
<i>E. Coli</i>	Negative/g	Negative	Negative	Negative
<i>S. aureus</i>	< 10 cfu/g	< 10 cfu/g	< 10 cfu/g	< 10 cfu/g
Salmonella	Negative/10 g	Negative	Negative	Negative
Heavy Metals				
Arsenic (inorganic)	≤ 1 ppm	< 0.42 ppm	< 0.45 ppm	0.04 ppm
Cadmium	≤ 1 ppm	< 0.1 ppm	< 0.11 ppm	< 0.1 ppm
Lead	≤ 1 ppm	< 0.1 ppm	< 0.11 ppm	0.38 ppm
Mercury	≤ 1 ppm	< 0.05 ppm	< 0.05 ppm	< 0.05 ppm
Pesticides and Toxins				
Microcystin Toxin	< 0.16 ppm (lod)	< 0.16 ppm	< 0.16 ppm	< 0.16 ppm
Organophosphorus Pesticides	None Detected	None Detected	None Detected	None Detected
Organochloride Pesticides	None Detected	None Detected	None Detected	None Detected
Pyrethroid Pesticides	None Detected	None Detected	None Detected	None Detected

lod=limit of detection

Heavy Metal Analysis

The CyaninPlus™ specification for heavy metals (inorganic arsenic, cadmium, mercury and lead) is ≤ 1 ppm. The results from three batch analyses for the presence of heavy metals, performed by Exova laboratory (Portland, Oregon) were shown previously in Table 2.

The specification for arsenic in CyaninPlus™ is based on inorganic arsenic levels, due to the known toxicity of inorganic forms (Jones 2007). Literature in the public domain (Kohlmeyer et al. 2002) as well as Desert Lake Technologies' in-house data on arsenic speciation, shows that inorganic arsenic species (As³⁺ and As⁵⁺) generally contribute less than 50 percent of the total arsenic measured in algae. Desert Lake Technologies performs arsenic speciation analysis (using ion chromatography and inductively coupled plasma mass spectrometry) on any batch of CyaninPlus™ that has a total arsenic level of > 1 ppm (as is the case in batch DLTL-01 93-3, where total arsenic=2 ppm and inorganic arsenic=0.04 ppm), to ensure that specifications for inorganic arsenic are met.

Residual Solvent Analysis

The only solvent that is used in the manufacturing of CyaninPlus™ is water; hence residual solvent analysis is not necessary and is not performed.

Residual Pesticide Analysis

A multi-residue pesticide analysis is performed on each batch of CyaninPlus™. Three batch analyses were shown previously in Table 2—batches were tested for residual pesticides by Exova laboratory (Portland, Oregon) for 164 pesticide compounds. No pesticide residues were detected in any of the batches.

Microcystins

Some cyanobacteria are capable of producing hepato- and neurotoxins. Microcystins are a group of monocyclic heptapeptides, which can cause morphological and functional changes in hepatocytes (Jiang et al. 2008). The World Health Organization (WHO) lists microcystin-LR (MC-LR) as one of the most abundant and one of the most toxic microcystins. As such they have provided guidelines for safe levels of MC-LR; these guidelines include a total daily intake of 0.04 µg/kg/bw and a provisional guideline value of 1 µg/L for drinking water.

Desert Lake Technologies tests for microcystin levels (many different variants, including MC-LR) in every batch of product as is shown in Table 2, to ensure that total levels are below the limit of detection, even though microcystins have not been documented in *Spirulina*. ELISA assays, with a limit of detection of < 0.16 ppm, were employed for the detection of microcystin toxins in three batches of CyaninPlus™. The results for all three batches of CyaninPlus™ products were < 0.16 ppm. These results indicated that no microcystin toxins are present in the tested batches at levels above the limit of detection.

Shelf-life Stability

The CyaninPlus™ specifications include a shelf-life of 36 months from the date of manufacture. A 36 month shelf-life is currently estimated based on two factors: 1) formal shelf-life studies performed by Desert Lake Technologies on another similar cyanobacteria (*Aphanizomenon flos-aquae*) showed a 36 month minimal shelf-life; and 2) phycocyanin that remains dry and stored away from light shows no degradation (in-house testing found no detectable degradation in material stored over 36 months).

Testing of five early batches of CyaninPlus™ showed that phycocyanin content, measured in mg/g, did not significantly change over a period of up to 24 months when stored under recommended storage conditions. These batches will continue to be monitored for phycocyanin content into the future. A formal accelerated shelf-life study is also planned for this ingredient.

Storage instructions for CyaninPlus™ are as follows: “Store in cool dry place, away from extreme conditions of heat, cold and moisture to prevent oxidation and preserve quality. Do not store this product above 100° F. Store product in an airtight container away from most sources of light and heat.”

Self-limiting Levels of Use

There are no specific self-limiting levels of use for CyaninPlus™.

Safety Assessment

Toxicology Studies

Numerous animal toxicological studies have been performed on *Spirulina* by various institutions around the world. These studies include acute, subchronic, chronic, mutagenic, teratogenic/developmental toxicity, carcinogenic, and multiple generational/reproduction studies. Such studies are pivotal in the determination that CyaninPlus™, a water extract of *Spirulina*, is safe. The pertinent studies are summarized in this notification. In addition, standard toxicological studies performed with phycocyanin are available in the public domain. The following review will include toxicological studies performed using both *Spirulina* species (*Arthrospira platensis* and *maxima*) as well as phycocyanin isolated from *Spirulina*.

As mentioned previously, phycocyanin constitutes 10–20% of the raw *Spirulina* material from which CyaninPlus™ is manufactured, as well as greater than 30% of the final CyaninPlus™ product. Based on the batch analyses reviewed, the average amount of phycocyanin in CyaninPlus™ was 44.2% (the values were 42.2%, 46.6% and 43.8% for three batches analyzed). The amount of phycocyanin present in harvested *Spirulina* has similarly been reported to range from 10–15%, as mentioned by Tomaselli and colleagues (Tomaselli et al. 1996). With this in mind, approximations on amount of phycocyanin in the *Spirulina* test articles, will be made at the end of each safety study discussed below—at 10%, 15% and 20%. This will help to demonstrate the equivalent phycocyanin exposure for each study.

Acute Oral Toxicity

Phycocyanin from *Arthrospira maxima* was tested in an acute toxicity study in OF₁ mice and Sprague-Dawley rats. The highest dose used in the study was 3 g/kg, given orally as one large bolus (gavage) at the beginning of the study (Romay et al. 1998). Mortality, body weight, and the behavior of the animals were recorded for 14 days after administration of the test article. Histopathological studies were performed on the highest dose group. The LD₅₀ was estimated to be greater than the highest dose tested, as there were no mortalities at this dose. No changes in behavior or histopathology were observed, nor were there any statistically significant effects on body weight. The publication lacks specific details with regard to study methods. In order to relate this study more specifically to phycocyanin, the phycocyanin equivalents, based on an estimated phycocyanin composition in *Spirulina* of 10–20%, are as follows:

- 10%—300 mg phycocyanin (3 g *Spirulina* group)
- 15%—450 mg phycocyanin (3 g *Spirulina* group)
- 20%—600 mg phycocyanin (3 g *Spirulina* group)

Hutadilok-Towatana and co-workers investigated the potential toxicity associated with *S. platensis* administered to male Swiss mice (30–36 g weight

range) over a 7-day acute oral toxicity study (Hutadilok-Towatana et al. 2008). The mice received either fresh (30 g/kg/bw) or dried (10 g/kg/bw) material and were sacrificed at the end of the study. Internal organ examinations revealed no treatment-related toxicological abnormalities. The phycocyanin equivalents of the dried test article in this study, based on an estimated phycocyanin composition in *Spirulina* of 10–20%, are as follows:

- 10%—1,000 mg phycocyanin (10 g dried *Spirulina* group)
- 15%—1,500 mg phycocyanin (10 g dried *Spirulina* group)
- 20%—2,000 mg phycocyanin (10 g dried *Spirulina* group)

Naidu and colleagues also performed an acute toxicity study on phycocyanin isolated from *Spirulina platensis* that was grown in raceway ponds, via acid extraction. Concentrations of 0.25–5.0 g/kg/bw were mixed into the basal diet of CFT-Wistar albino rats and fed for a single day. The control group consisted of rats fed the basal diet alone. Rats were randomly divided into six groups, with eight rats per group. The animals were observed for 21 days and then were sacrificed. Vital organs were dissected, weighed and histopathologically examined. Acute treatment with phycocyanin did not induce mortality or any symptoms of toxicity. Therefore, the dietary LD₅₀ of phycocyanin in this study was greater than 5.0 g/kg/bw (Naidu et al. 1999).

Sub-Chronic Oral Toxicity

Naidu and colleagues performed a subchronic study on low doses of phycocyanin, isolated from *S. platensis*, in CFT-Wistar albino rats (Naidu et al. 1999). Forty rats were divided into five groups of eight rats per group. The groups received either basal diet alone (control), phycocyanin at 0.5, 1.0, 2.0 or 4.0 g/kg of their daily diet for 14 weeks. (Note the units are g/kg of the diet, and not g/kg body weight). Daily food intake and body weights were recorded. After 14 weeks, the rats were necropsied. Organ weights were recorded and histopathological examination was performed. Hematological and serum chemistry analyses were also performed using blood collected by heart puncture. There were no significant deviations in food intake, body weight gain, organ weights, hematological or serum chemistry measurements in treated rats when compared to controls. The authors state “total concentrations of phycocyanin consumed during the study based on a mean of 90 days on 4 g/kg of phycocyanin in the diet was calculated to be 17 g/kg/bw and 21.3 g/kg/bw of phycocyanin per day for male and female rats, respectively”. This is assumed to be based upon the total diet consumed over the course of the study. The authors also report that male rats in the high dose group consumed a mean of 10.5 g diet/day and females consumed 9.44 g diet/day. This can be calculated as a daily consumption of 42 mg and 38 mg of phycocyanin per day for male and female rats, respectively. However, the concentration of phycocyanin consumed daily per kg body weight was not reported by the authors. If we use an average weight for the rats as 200 g, the high dose calculates to a No Observable Effect Level (NOEL) of 190 mg/kg bw/day phycocyanin (females). However, this is an estimation because the authors did not report a NOEL (Naidu et al. 1999).

Hutadilok-Towatana and co-workers conducted a 12-week sub-chronic toxicity study of *S. platensis* in female Spargue-Dawley rats, aged 5 weeks (Hutadilok-Towatana et al. 2008). Rats of each sex were randomly divided into 4 groups (n=6) for treatment with either fresh or dried *S. platensis*: (1) fresh *S. platensis* was orally administered at 0, 300, 600 and 1,200 mg/kg/ bw; (2) dried *S. platensis* was orally administered at 0, 30, 60 and 120 mg/kg/ bw. During the 12-weeks, daily observation (including food and water uptake) and body weight gains revealed no abnormal clinical, physical or behavioral changes. No significant changes in clinical chemistry or hematological parameters surfaced. A small increase in hematocrit values was observed for treated animals, but was not dose-dependant. The authors reported a decrease in lymphocyte ratios for treated animals, and stated that this decrease coincided with an increase in neutrophil and monocyte values:

Dose Level (mg/kg/bw)	0	300	600	1200
Female rats—Neutrophil values (%)	0.33	2.00	10.80	11.50
Male rats—Neutrophil values (%)	1.33	2.20	6.60	15.33

The authors mentioned that this apparent dose-dependant neutrophil increase could be the result of the long-term exposure to *S. platensis*. Terminal examinations revealed no signs of infection or inflammation, which confirms that the increase in neutrophil count is unlikely due to an underlying infection. No macroscopic organ abnormalities were observed in any of the animals, and no gastrointestinal abnormalities, such as lesions, resulted from the long-term exposure to *S. platensis*—fresh or dried. The phycocyanin equivalents of the maximum dried Spirulina level, based on an estimated phycocyanin composition in Spirulina of 10–20%, are as follows:

- 10%—12 mg phycocyanin (120 mg Spirulina group)
- 15%—18 mg phycocyanin (120 mg Spirulina group)
- 20%—24 mg phycocyanin (120 mg Spirulina group)

In 1980, the United Nations Industrial Development Organization (UNIDO) performed comprehensive safety testing of Spirulina in animals. The species of Spirulina that was used was not mentioned, but was most likely *S. maxima*, due to the manufacturer—Sosa Texcoco). In the sub-chronic study, Wistar rats were divided into five groups of 10 rats/sex/group, and were fed a diet of 10, 20, and 30% Spirulina in place of soy for a period of 13 weeks. The control groups consisted of a soy-based diet group and a commercial diet (commonly used in the laboratory) group. The authors concluded that Spirulina did not affect any of the parameters studied. No statistically significant changes occurred in any of the parameters recorded with the exception of an increase in the relative weights of the seminal vesicles of animals treated with 20 and 30% Spirulina. The authors concluded that this was not of toxicological interest or concern because there were no pathological findings in the seminal vesicles upon the histopathological examination (Chamorro-Cevallos 1980). The phycocyanin equivalents of the maximum test level, based on an estimated phycocyanin composition in Spirulina of 10–20%, are as follows:

- 10%—3% phycocyanin in the diet (30% Spirulina group)
- 15%—4.5% phycocyanin in the diet (30% Spirulina group)
- 20%—6% phycocyanin in the diet (30% Spirulina group)

Chronic Oral Toxicity

Yang and co-workers assessed the safety of *S. platensis* in male and female mice (C57BL/6J), aged 4 weeks, in a 6-month study (Yang et al. 2011). Three *S. platensis* dose levels at 0%, 2.5% and 5% by weight, with n=8, were investigated in which powdered *S. platensis* was added to the animal feed. During the treatment period, the mice were observed for mortality, morbidity and behavior. Animals were weighed biweekly, and blood samples were collected at 2, 4 and 6 months and analyzed for alanine aminotransferase (ALT) and aspartate aminotransferase (AST). At treatment termination animals were euthanized and livers were weighed. No deaths, illness or behavior related changes were observed in any of the animals during the treatment period. All animals exhibited normal growth patterns and no observed adverse treatment related side effects were observed. ALT levels for all animals were consistent with that of the controls at all the tested time intervals. However, males in the 5% *S. platensis* group had lower ALT plasma levels during the second month's examination. No elevated ALT values were observed during the study. Male rats in both the 2.5% and 5% *S. platensis* groups and females in 5% *S. platensis* group had lower AST levels at the 6-month sample period. The authors stated that all animals in the control groups had elevated AST levels (a two-fold increase) by the end of the study, and hypothesized that the observed decrease seen in the treated animals could be due to the Spirulina's ability to prevent age-related tissue damage. Histopathological examinations at the end of the study revealed "mild to moderate lipidoses" for all animals, including those in the control groups. However, the researchers stated that no liver damage or signs of hepatitis were detected in any of the animals. The phycocyanin equivalents of the maximum dried Spirulina level, based on an estimated phycocyanin composition in Spirulina of 10–20%, are as follows:

- 10%—0.5 % phycocyanin in the diet (5% Spirulina group)
- 15%—0.75 % phycocyanin in the diet (5% Spirulina group)
- 20%—1 % phycocyanin in the diet (5% Spirulina group)

UNIDO also conducted a chronic oral toxicity study of Spirulina. As in the sub-chronic study, Wistar rats were fed a diet of 10, 20, and 30% Spirulina in place of soy, for a period of 80 weeks. Control groups received a soy-based diet or a commercial diet commonly used in the laboratory. Each of the five groups consisted of 20 rats of each sex. The authors concluded that Spirulina did not produce any toxic effects in any of the parameters studied including weight gain, hematological parameters, liver and kidney function, terminal serum chemistry, mortality, relative organ weights, and histopathological parameters including tissue lesions and tumor incidence (Chamorro-Cevallos 1980).

Reproductive/Developmental Toxicity

Kapoor and Mehta investigated the effects of *S. platensis* supplemented diets in Albino rats (Kapoor et al. 1993). Five diets were examined: (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), and (5) *S. platensis*—without additional vitamins and minerals (480 g/kg). Additional vitamin and mineral supplements were added to diets 1–4. In comparison, rats on the *S. platensis* diet devoid of minerals and vitamins had significantly lower food intake, and rats on the wheat gluten diet had the lowest pregnancy weight gain. The highest pregnancy weight gain was recorded for rats on the *S. platensis* + wheat gluten diet. The number of pups born per group was higher in the *S. platensis* groups, and the total litter weights were also higher in these groups. The authors stated that the relatively high vitamin E content of *S. platensis* (19 mg/100 g) might have played a role in the litter sizes and concluded that Spirulina appears to be a good dietary supplement during pregnancy. The hematological parameters for pregnant females indicated higher iron storage and hemoglobin values for females on any of the *S. platensis* diets. However, on day 20 of the study a decrease in iron storage and serum iron were pronounced in *S. platensis* fed rats, and the authors stated that this could be due to the greater iron demand from larger litter sizes associated with the *S. platensis* fed rats. The outcome of the study suggests that *S. platensis* diets could enhance pregnancy by increasing the weight gain during pregnancy, increasing litter sizes and promoting healthy iron levels during pregnancy. The phycocyanin equivalent dose for the *S. platensis* group (group 2), is shown below:

- 10%— 48 g/kg phycocyanin in diet (480 g/kg Spirulina group)
- 15%— 72 g/kg phycocyanin in diet (480 g/kg Spirulina group)
- 20%— 96 g/kg phycocyanin in diet (480 g/kg Spirulina group)

UNIDO sponsored a multigenerational toxicology study of Spirulina to investigate any effects this ingredient may have on reproduction and lactation. The three-generation (two litters per generation) study took place over a two-year period, and the last generation was subjected to a conventional sub-chronic toxicity study (Chamorro-Cevallos 1980). Wistar rats were divided into five groups of five animals/sex/group, and were fed 10, 20 and 30% Spirulina or one of two control diets (a commercial diet or a soy-based diet). Fertility, gestation, lactation, and viability indices were recorded. For the 13-week sub-chronic part of the reproduction study, 10 males and 10 females were randomly selected from the F3b generation. The general condition, weight increase, food consumption and conversion efficiency, hematology, serum and urine analyses, and organ weight were recorded and histopathological examination of the organs was performed.

In the first generation, the fertility index was similar in all groups, and no negative effects from Spirulina administration related to gestation index were observed. There were no changes in the viability or lactation indices of the litters that were related to treatment. Mean weights of the litters matched the controls. Viability and lactation index results corresponding to the second mating of this generation were reduced in relation to results of the first matings but were

similar to controls. The litter weights of this generation were also similar to the controls. While the lactation index for this group was slightly reduced compared to the first mating, the litter growth during weaning was the same for the Spirulina and control groups. The results of the second mating of the F1b generation and the F2b generation showed no variations between the groups. Overall, no effects on fertility, litter size, or mortality were observed.

The results of the 13-week subchronic study performed with the third generation (F3b) groups found significant differences in specific gravity of urine in the male group receiving 20% Spirulina, and in the females in the commercial diet control group (as opposed to the soy control group). The effects observed on urine specific gravity were considered unrelated to treatment because the effect in males was observed only in the mid-dose group and the effects in females occurred in one of the control groups compared to the other control group. Significant differences were also noted in the weights of the heart (20% group), kidneys (30% group), and seminal vesicles (20 and 30% groups) of the males and in the weights of the lungs (30% group) and spleens (20% group) of females. The differences were not always dose-dependent and were not accompanied by any pathological differences that could be attributed to Spirulina and therefore were considered unrelated to treatment. Macroscopic examination revealed hydronephrosis in all groups, but no histopathologically examined lesions were attributed to Spirulina, including in the kidney.

It is worth mentioning that a 13-week sub-chronic study by Salazar et al. in 1998 on the safety of *S. maxima* in mice, also reported increased seminal vesicle weights for male mice receiving *S. maxima* supplemented diets (Salazar et al. 1998). However, the authors stated that all other organs weights were similar to those of the control animals and that macroscopic organ examinations revealed no organ abnormalities as a result of *S. maxima* treatment. Furthermore, no reproductive and developmental abnormalities were observed in the reproductive study described above.

The UNIDO organization performed teratogenicity/developmental toxicity studies to detect any Spirulina-related effects such as embryonic resorptions or fetal malformations (Chamorro-Cevallos 1980). Wistar rats, CD-1 mice, and Dorado hamsters born to mothers fed 10, 20, and 30% Spirulina-containing diets were studied, along with two control groups as in the previous UNIDO-sponsored studies. Spirulina was fed to the treatment groups over three different periods during gestation: rats gestation days 7 to 9, days 7 to 14, days 1 to 14, or days 1 to 21; mice gestation days 7 to 8, days 7 to 13, days 1 to 13, or days 1 to 19; and hamsters gestations days 7 to 9, days 7 to 11, days 1 to 11, or days 1 to 14. After the pregnant animals were weighed and sacrificed, fetuses were removed by caesarean section, counted, weighed, and examined for internal (visceral and skeletal) or external malformations. The number of implantation sites was counted in each dam. 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 fetuses, the number of implantations per fertile female, and the number of fetuses per pregnant female, but they did not show any relationship to the dose of Spirulina fed to the animals. The authors' overall conclusion was that

Spirulina does not cause fetal malformations, anomalies, or resorptions (Gershwin and Belay 2008). The phycocyanin equivalents used in the UNIDO studies, at the highest dose level (30%), based on an estimated phycocyanin composition in Spirulina of 10–20%, are as follows:

- 10%—3% phycocyanin in the diet (30% Spirulina diet group)
- 15%—4.5% phycocyanin in the diet (30% Spirulina diet group)
- 20%—6% phycocyanin in the diet (30% Spirulina diet group)

Human Studies

Spirulina has been used in a number of human studies that measured particular safety parameters. These studies aid and corroborate the excellent safety profile of Spirulina consumption, and subsequently, of the aqueous extract CyaninPlus™, in part by confirming the absence of adverse effects. Some pertinent human studies that investigated parameters relevant to safety are summarized below.

Fifteen overweight patients participated in a randomized, double-blind, placebo-controlled crossover study of Spirulina (Becker et al. 1986). Subjects consumed 2.8 g of Spirulina three times daily for four weeks and experienced no adverse effects on blood pressure, heart rate, complete blood count, blood chemistry markers, kidney function, enzyme activities, or physical symptoms.

Thirty men with hypercholesterolemia and mild hypertension were given 4.2 g of Spirulina per day for either eight weeks, or four weeks followed by four additional weeks of observation. No significant changes occurred in white blood cells, lactate dehydrogenase, glutamate oxaloacetate transaminase, gamma-glutamyl transpeptidase, glutamic-pyruvate transaminase, alkaline phosphatase, uric acid, blood urea nitrogen, or creatine. No adverse effects were reported by any of the subjects and no problems were found on clinical examination (Nakaya et al. 1988).

Spirulina was also well tolerated for at least 150 days when given to 5,000 Indian rural preschool children (1 g per day) in a Shri AMM Murugappa Chettiar Research Center- sponsored program (Seshadri 1993; Simpure et al. 2006).

Immunological Studies

Park and co-workers investigated the effects of Spirulina in 78 Korean males and females, aged 60–87 years, (Park et al. 2008). During the four months, participants took either 8 g Spirulina per day or the placebo (starch pills) in the absence of any other medications or supplements. Baseline parameters including weight, height, body fat and vital signs were collected prior to the treatment along with baseline blood sugar, triglycerides, cholesterol and plasma immune parameters (IL-2, IL-6, TNF- α and MCP-1).

Two immunological parameters showed statistically significant differences between the treatment groups. First, the researchers reported an increase in plasma IL-2 levels from baseline for males receiving Spirulina pills, while males in the placebo group showed no significant change in their IL-2 levels:

	Spirulina group Baseline vs. 4 Month	Placebo group Baseline vs. 4 Month
Males IL-2, pg/mL	9.43 vs. 13.6*	13.2 vs. 13.0
Females IL-2, pg/mL	9.36 vs. 13.8*	10.9 vs. 13.3*
Males IL-6, pg/mL	2.64 vs. 1.94	1.07 vs. 1.99
Females IL-6, pg/mL	1.02 vs. 1.80*	1.52 vs. 1.65

* $p < 0.05$

Females on the other hand showed increased IL-2 levels in both the treatment and placebo groups. Interestingly, at the end of the 4-month study, both Spirulina treated male and female IL-2 values were comparable to that of the control individuals. The second difference was in the IL-6 values. Spirulina treated males had a decrease in IL-6 values, but these values compared to that seen in placebo group individuals. Spirulina treated females showed an increase in IL-6 values, but again, these values compared to what was observed for the placebo treated individuals. All other immune parameters tested showed no significant change.

The findings of this study suggest that Spirulina supplementation in this specific population (elderly Korean individuals) altered IL-2 and IL-6 levels from baseline values. However, the final test results showed no difference between the Spirulina and placebo individuals. Furthermore, although an increase in IL-2 may raise concerns in certain sub-populations, such as those on immunosuppressant drugs, it is necessary to point out that a *normal* IL-2 level is < 35 pg/mL according to the Mayo Medical Laboratory (ELISA testing), and their reference for IL-6 is < 17.4 pg/mL.

There are several literature examples supporting *normal* IL-2 values in human control groups to be < 35 pg/mL and that of IL-6 to be < 17.4 pg/mL. In the first example, human IL-2 values come from the 2004 publication by Ceyhan and co-workers conducted in Turkey (Ceyhan et al. 2004). Researchers investigated the IL-2 and IL-10 levels in asthma patients compared to healthy control group subjects. Their control group consisting of 5 individuals (2 males and 3 females, average age 30 years), and their serum IL-2 values were determined to be 30.3 pg/mL using the same ELISA assay used by Park *et al.* (IL-2 Quantikine, R&D Systems Inc. USA).

In the second example, Takahashi and co-workers investigated the serum cytokine and growth factors in Japanese patients with psoriasis (Takahashi et al. 2010). In this study, the researchers reported the IL-2 level in the control group, consisting of 78 individuals (aged 24–76 years) to be 16.8 pg/mL. The IL-6 values were 1.81 pg/mL, similar to what was reported in the Park study. In this experiment the ELISA assay kit used was from Biosecure, Camarillo, CA, USA.

Tuncer and co-workers investigated the serum cytokine levels in non-alcohol fatty liver subjects (Tuncer et al. 2003). The average IL-6 level for their control group, consisting of 30 healthy individuals, was 2.55 pg/mL.

Hence final values for IL-2 and IL-6 in the Park *et al.* study are within values considered well within the normal range.

On a final note, Mao and co-workers investigated the effects of Spirulina on cytokine production in 36 individuals with allergies, aged 18–55 years (Mao et al. 2005). In this study 36 patients were divided into three groups, a placebo group,

a 1.0 g Spirulina/day group and a 2.0 g Spirulina/day group. After the 12-week treatment period no difference was observed in the IL-2 levels in any of the three groups.

In conclusion, although the Park study indicated an increase in IL-2 values associated with consumption of 8.0 g of Spirulina, the recorded serum IL-2 levels compared to those seen in healthy human subjects in other studies. Furthermore, at the end of the study, the Park study reported no difference between the IL-2 values in subjects receiving Spirulina compared to subjects receiving the placebo. Lower doses of Spirulina, such as in the Mao study, did not lead to any changes in IL-2 levels. Hence consumption of up to eight grams per day of Spirulina in certain populations where immunostimulation might be undesirable will not be of concern. It is unclear what specific constituents of Spirulina have immunomodulatory properties.

History of Consumption

Spirulina has a documented history of use as a food for more than 1,000 years. As stated earlier, Spirulina, specifically *S. maxima*, was reported as being consumed as a food as far back as 1521, where the alga was harvested from Lake Texcoco in Mexico (Ciferri 1983; Gershwin and Belay 2008). An early account of the consumption of Spirulina in Africa came from an account in the Republic of Chad, in 1940, where an alga, later identified as *S. platensis*, was harvested from Lake Chad and eaten by the native population (Ciferri 1983; Gershwin and Belay 2008).

Spirulina has been in production as a supplement for over 30 years (Yoshikawa and Belay 2008). More than 3,000 tons of *A. platensis* are produced annually worldwide, the majority of which is used in health food products and in animal feed (Eriksen 2008). Numerous food and dietary supplement products are sold today that contain Spirulina. There are also dietary supplements on the market that contain specified levels of phycocyanin.

C-phycocyanin from *A. platensis* is also sold as a food and cosmetics colorant in Japan, and has been authorized for use in products such as gums, candies, fermented milk products, ice cream, soft drinks, milk shakes and dairy products (Naidu et al. 1999; Eriksen 2008; Silveira et al. 2008).

Information that may appear to be Inconsistent with GRAS Determination

Spirulina has a long history of use as a food source. There have been reports of rare adverse incidences associated with consumption of Spirulina, all of which are single-case events. These reports are summarized below. When one considers the vast amount of Spirulina that is consumed throughout the world, these reports are extremely rare and do not raise safety concerns.

In 2008 a 28-year-old man developed rhabdomyolysis after taking Spirulina (3 g *A. platensis* per day as a dietary supplement) for one month (Mazokopakis et al. 2008). He was not taking any other medications, and did not use alcohol, cigarettes or illicit drugs. The symptoms were relieved after discontinuing the supplement. Spirulina supplementation was suspected to be the cause, since

other causes were excluded. No other reports of Spirulina-related rhabdomyolysis have been reported in the literature to the best of our knowledge. In contrast to this single case report, a small clinical study published in 2006 showed that Spirulina exhibits potential preventive effects related to exercise-induced skeletal damage (Lu et al. 2006).

In 2010, Petrus *et al* reported on an anaphylactic reaction in a 14-year-old adolescent after taking five Spirulina tablets—no dose is mentioned (Petrus et al. 2010). The adolescent experienced urticaria, labial edema and asthma six hours after consumption of the supplement. He had a positive prick test and oral challenge test to Spirulina. The IgE-reactive fractions from an extract of the Spirulina tablets were identified by MALDI-TOF analysis as the β -chain of c-phycocyanin. This is the only reported allergenic reaction to Spirulina and/or phycocyanin in the public domain to the best of the authors' knowledge, and hence appears to be an extremely rare occurrence based on the high consumption rate of Spirulina throughout the world. Additionally, in 1997 Yang and co-workers investigated the anaphylactic inhibition properties of *S. platensis* in rats subjected to compound 48/80 (8 μ g/g/bw), which induces histamine secretion (Yang et al. 1997). Researchers reported that 0.5 and 1.0 mg/g/bw *S. platensis* actually inhibited systemic anaphylaxis resulting in 0% mortality—in contrast to the control group, which had 100% mortality. The authors also tested the inhibition ability of *S. platensis* on IgE-mediated anaphylaxis and found 0.5 and 1.0 mg *S. platensis*/g/bw to have 40.8% and 68.7% inhibition rates, respectively.

Lastly, in 2002 a 52-year-old Japanese man with a history of ill health and taking several medications, showed signs of liver damage after taking an unknown dose Spirulina for five weeks (Iwasa et al. 2002). The authors stated that his signs resolved after discontinuing all medications and Spirulina supplements. This case study is mentioned as it is in the public domain; however it is unclear if the liver damage was due to consumption of Spirulina. This is the only case of potential Spirulina-induced hepatotoxicity reported in the literature to the best of the authors' knowledge.

In summary, although isolated single case adverse events have been associated with Spirulina consumption, including rhabdomyolysis, allergenic potential and hepatotoxicity, in all but the allergenic reaction, the true cause of the symptoms was not proven. Furthermore, research evidence suggests that Spirulina inhibits anaphylaxis rather than causes it, and benefits skeletal muscle rather than harms it. Lastly, compared to the worldwide consumption levels of Spirulina, this small handful of case studies does not cause any safety concern with regard to general consumption of Spirulina or CyaninPlus™.

Current Regulatory Status

In 2003, Cyanotech Corporation and Earthrise Nutritionals, Inc. notified FDA that their dried biomass of *A. platensis* is GRAS, through scientific procedures, for use as an ingredient in foods such as specialty food bars, powdered nutritional drink mixes, popcorn, and as a condiment in salads and pasta, at levels ranging from 0.5 to 3 g per serving (GRAS Notice No. GRN 000127). The notifiers estimated that a high end user would consume up to 6 g per day of *A. platensis*. A

low end user was estimated to consume 3–12 g per month. FDA filed the GRAS notification without questions.

Spirulina is also recognized as a food by government organizations. For example, the Convention for the Use of Food Micro-Algae, Intergovernmental Institutional Spirulina Program (CISRI-ISF/ IIMSAM) works to promote the use of Spirulina against severe malnutrition. The Food and Agriculture Organization of the United Nations considers Spirulina an important human food, rich in macro- and micronutrients (Habib et al. 2008). The National Aeronautics and Space Administration (NASA) and the European Space Agency (ESA) recommended Spirulina as one of the primary foods during long-term space missions (Habib and Parvin 2008; Deng et al. 2010). Lastly, the USDA National Nutrient Database for standard reference, which provides information for most food composition databases in the public and private sectors, lists nutrient data for dried Spirulina, suggesting that it is considered a food by this organization.

Regulation of Color Additives

While CyaninPlus™ has a deep blue color and may impart color to food, its intended use with regard to this GRAS determination is not as a color additive. Color additives are required to undergo premarket review and approval by FDA prior to being sold for that purpose. A color additive is defined in 21 CFR 70.3 as “any material, not exempted under section 210(t) of the act, that is a dye, pigment, or other substance...that, when added or applied to a food, drug, or cosmetic or to the human body or any part thereof, is capable (alone or through reaction with another substance) of imparting a color thereto....Food ingredients...which contribute their own color when mixed with other foods are not regarded as color additives;...but [if a] food is deliberately used as a color, it is a color additive.”

21 CFR 70.3(g) also states that an ingredient can be exempt from being called a color additive on the basis that its intended use is solely for purposes other than coloring, and that any color imparted is clearly unimportant insofar as the appearance, value, marketability, or consumer acceptability is concerned. (It is not enough to warrant exemption if conditions are such that the primary purpose of the material is other than to impart color.)

CyaninPlus™, manufactured by Desert Lake Technologies LLC, is not intended for use as a color additive for the purposes of this GRAS notification. It is hence exempt from FDA premarket approval as a color additive, as long as when it is added to a product, any color that results is clearly unimportant to the product's appearance, value, marketability, or consumer acceptability.

Intended Use and Estimated Daily Intake (EDI)

CyaninPlus™ is a water extract of Spirulina (*A. platensis* and/or *A. maxima*). The extraction process results in a final ingredient that contains greater than 30% c-phycoerythrin, as compared to the Spirulina starting material, which contains 10–20% c-phycoerythrin. Batch analyses displayed in this notification show that a typical batch of CyaninPlus™ contains 42–47% c-phycoerythrin.

For the purpose of this GRAS notification, Desert Lake Technologies' CyaninPlus™, manufactured in accordance with current Good Manufacturing Practices (cGMP), is intended to be used as an ingredient in food. CyaninPlus™ is not intended for use in infant formula, meat, egg, catfish or any products that would require additional regulatory review by USDA.

Because CyaninPlus™ is a water extract of *Spirulina* and is not chemically modified, toxicological studies on *Spirulina* are pertinent and pivotal in supporting the safety of this ingredient. Toxicological studies in animals show that consumption of *Spirulina* is safe at high levels (up to 30% of the diet in the UNIDO studies on *S. maxima*, and 5% to nearly 50% of the diet in the Yang and Kapoor studies, respectively, on *S. platensis*). Many organizations are working to increase consumption levels of *Spirulina* around the world, due to its desired macro- and micronutrient profile (Habib and Parvin 2008).

CyaninPlus™ is intended to be used in foods as a food ingredient at an addition level up to a maximum of 250 mg per serving, equivalent to approximately 125 mg of phycocyanin per serving. In order to calculate an estimated daily exposure level for this ingredient, data reported in an article from the USDA Center for Nutrition Policy and Promotion was utilized (Basiotis et al. 2000). USDA utilized data from Market Research Corporation of America Information Services, on 5,752 adults for the 1992–1994 period, as relates to their consumption of foods based on detailed 14-day food diaries. According to the data, males aged 51 or greater consumed the greatest total number of servings of food from all food groups (18.2 total servings per day). Women aged 19–24 consume the least number of servings of all food groups (12.5 total servings per day).

If 10% of a person's daily food servings (based upon the USDA maximum described above) contained CyaninPlus™, then an individual would consume 1.82 servings of CyaninPlus™ per day, at a maximum of 250 mg per serving. That is equivalent to 455 mg CyaninPlus™ per day, or approximately 228 mg of c-phycocyanin per day. If 50% of an individual's total servings of food per day (9.1 servings per day based upon the USDA maximum described above) contained CyaninPlus™, the resulting accumulative daily exposure to CyaninPlus™ at the maximum addition level of 250 mg per serving would be 2.28 grams, or approximately 1.14 grams of c-phycocyanin per day.

Based on the *Spirulina* starting material, which contains up to 20% c-phycocyanin, consumption of approximately 6 grams per day of *Spirulina* would result in an exposure to 1.2 grams of c-phycocyanin. The exaggerated daily consumption level described above (9.1 servings per day CyaninPlus™) as it relates to daily c-phycocyanin exposure is still consistent with the GRAS standard of "reasonable certainty of no harm", based on scientific procedures.

Exposure to 6 grams per day of *Spirulina* is also consistent with previous GRAS notification number 000127, which estimated that a high end user would consume up to 6 grams per day of *A. platensis*. Additional components that accompany phycocyanin during the aqueous extraction process are also considered safe as components of *Spirulina*.

The addition of CyaninPlus™ to food categories is intended to replace consumption of other forms of *Spirulina* added to foods in the same respective

food categories. For example, if a consumer were to choose a beverage containing CyaninPlus™, this would likely replace consumption of a beverage or other food product that contains Spirulina or Spirulina extracts. Hence, for consumers who already purchase food products containing Spirulina, CyaninPlus™ is not expected to add additional exposure to Spirulina or its components from the same categories of foods.

General Recognition

Toxicological safety assessments on both Spirulina and phycocyanin have been published and are available in the public domain, and suggest no toxicological concern with regard to consumption of Spirulina, phycocyanin and/or CyaninPlus™. The publicly available safety information related to CyaninPlus™, that is the basis for this GRAS determination, meets the requirement for common knowledge and general recognition. It shows that there is consensus among qualified experts that the ingredient is generally recognized as safe for its intended use as stated in this notification. Citations for these assessments can be found in the reference section of this dossier.

Basis for the GRAS Determination

CyaninPlus™, a water extract of Spirulina (*A. platensis* and/or *A. maxima*), has been the subject of a thorough safety assessment as described above. CyaninPlus™ is manufactured via a water extraction manufacturing process that does not chemically alter the extract and does not present safety concerns. Batch analyses of CyaninPlus™ show production consistency that meets all product specifications. Animal and clinical scientific studies support the safety of Spirulina (both *A. platensis* and *A. maxima*) as well as phycocyanin, and these scientific studies are supported by a long history of safe consumption.

As discussed in the Safety Assessment section of this notification, acute oral toxicity, sub-chronic oral toxicity and chronic oral toxicity studies performed on Spirulina are pivotal for assuring that CyaninPlus™ at its intended use level is safe for consumption and free of toxicological effects. The long history of human consumption, as well as governmental and non-profit organizations' positions on the use of Spirulina as a food corroborates the fact that Spirulina, and hence CyaninPlus™, is safe for human consumption. Furthermore, *A. platensis* is the subject of GRN 000127, which was filed with the FDA in 2003 without comment. With exception to the small handful of case-reports on allergic or other events discussed previously, no reports of adverse events associated with the long-term consumption of Spirulina have been found in the public domain. Based on the evidence provided in this notification, CyaninPlus™ is considered GRAS for its intended use in food.

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