GR

Orginial Submission

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Prepared and submitted by:
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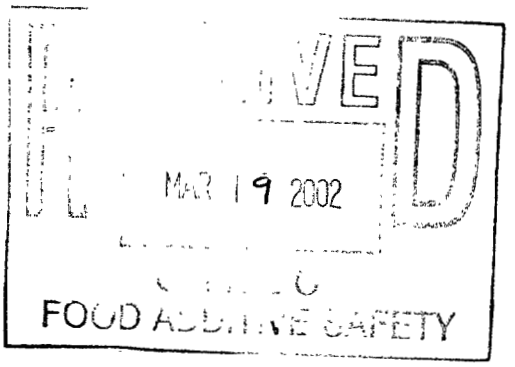


Notifiers:
Cyanotech Corporation
73-4460 Queen Kaahumanu Hwy.,
#102
Kailua-Kona, HI 96740

Earthrise Nutritionals, Inc.
424 Payran Street
Petaluma, CA 94952

Re: GRAS Notification: *"Spirulina"* microalgae

March 8, 2002

Office of Premarket Approval (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C Street, S.W.
Washington, D.C. 20204

Dear CFSAN Agency,

Per proposed Section 21 CFR 170.36, Cyanotech Corporation (Kailua-Kona, HI) and Earthrise Nutritionals, Inc. (Petaluma, CA) hereby notify the Food and Drug Administration that they claim the use of "Spirulina microalgae" in certain food products is exempt from premarket approval requirements of the Federal Food, Drug and Cosmetic Act because the notifiers have determined that such use is GRAS. The basis for such determination is by scientific procedures. Cyanotech Corporation and Earthrise Nutritionals, Inc. each accept responsibility for this GRAS determination.

Spirulina has a long history and documented record of human consumption, and is known in the literature and by prominent researchers as a safe and nutritious microalgae. In the past 20 years, it has been marketed and consumed as a safe human food by millions of people in North and South America, Asia, Europe and Africa. Furthermore, *Spirulina* has been approved as a food for human consumption by many governments, health agencies and associations of over 60 countries. Based on 30 years of safety and quality research, many countries and organizations have established *Spirulina* quality and safety standards, which are abided to by Cyanotech Corporation and Earthrise Nutritionals, Inc. *Spirulina* can be cultivated under scientifically controlled conditions that virtually eliminate contamination by other algae.

Numerous data published in the primary published scientific literature, including work with malnourished children, does not raise any safety issues with *Spirulina* and thereby provides abundant evidence that there is a consensus among qualified experts, including the United Nations Food and Agriculture Organization (UNFAO) and United Nations Industrial Development Organization (UNIDO), that there is reasonable certainty that the substance is not harmful under the intended conditions of use. Cyanotech Corporation and Earthrise Nutritionals, Inc. also have several toxicity data that demonstrate the safety of *Spirulina* for its intended use.

Considering the totality of other corroborative evidence, the safety studies with animals and humans demonstrate there is a reasonable certainty that *Spirulina* is not harmful under the intended conditions of use. Toxicity studies in both animals and humans demonstrate no adverse affects. Furthermore, according to the US FDA, *Spirulina* can be legally marketed in the US as a food as long as it is labeled accurately and contains no contaminated or adulterated substances (Talk Paper, 6/23/82).

The common name of this substance is *Spirulina* microalgae. The intended use of the substance is as a source of protein and phytonutrients, such as carotenoids. *Spirulina* would be added to limited categories of food. The first category is that of the specialty granola/breakfast/power/energy bars and the second is powdered nutritional drink mixes such as "smoothies". Additionally, the Notifiers propose the use of *Spirulina* as a condiment for salads and pasta. The level of use for these foods would be from 0.5-3 grams of *Spirulina* per serving size. The population expected to consume these foods are those that are active, healthy and usually exercise on a regular basis. Vegetarians have long been typical and traditional consumers of *Spirulina*. The substance is not intended for infant formulas.

Although *Spirulina* is a blue-green algae, the intended use is not as a color additive and similar to other approved GRAS substances such as Brown Algae (21 CFR 184.1120) and Red Algae (21 CFR 184.1121). Furthermore, we claim that *Spirulina* is not a color additive per 21 CFR 70.3 (f) as it is a food ingredient such as cherries, green or red peppers or chocolate that contributes it's own natural color when mixed with other foods. The intended use of *Spirulina* microalgae is not similar to beet extract used to color lemonade a pink color.

Also enclosed in triplicate, are references that are discussed in the accompanying package. Other data and information that are the basis for the notifiers' GRAS determination

are available for FDA review and copying at reasonable times at the above addresses or will be sent to FDA upon request. Please find the attached information and data for your review per 62 FR 18937 (April 17, 1997) and proposed 21 CFR 170.36.

Detailed information such as the identity of the notified substance, quantitative composition, method of manufacture, source information, characteristic properties, specifications for food-grade material and possible contaminates of *Spirulina* follows in the package that accompanies this letter.

Please be advised that a new Authorized Official will be administrating this GRAS Petition. Dr. John Dore has recently been appointed by Cyanotech as Scientific Director and will be fulfilling all future concerns with the petition. If you should have any questions or require further information, please do not hesitate to contact him.

His contact information is:

Dr. John Dore
Scientific Director
Cyanotech Corporation
73-4460 Queen Kaahumanu Hwy., #102
Kailua-Kona, HI
96740
tel: 808-326-1353
fax:808-329-3597
email: jdore@cyanotech.com

Sincerely,

R. Todd Lorenz, Ph.D.
For Cyanotech Corporation & Earthrise Nutritionals, Inc.

GRAS Notification for *Spirulina* Microalgae

(1) Exemption Claim:

Per proposed Section 21 CFR 170.36, Cyanotech Corporation (Kailua-Kona, HI) and Earthrise Nutritionals, Inc. (Petaluma, CA) hereby notify the Food and Drug Administration that they claim the use of *Spirulina* microalgae in certain food products is exempt from premarket approval requirements of the Federal Food, Drug and Cosmetic Act because the notifiers have determined that such use is GRAS. The basis for such determination is by scientific procedures. Cyanotech Corporation and Earthrise Nutritionals Inc. equally accept responsibility for this GRAS determination.

Although *Spirulina* is a blue-green algae, the intended use is not as a color additive and similar to other approved GRAS substances such as Brown Algae (21 CFR 184.1120) and Red Algae (21 CFR 184.1121). Furthermore, we claim that *Spirulina* is not a color additive per 21 CFR 70.3 (f) as it is a food ingredient such as cherries, green or red peppers or chocolate that contributes it's own natural color when mixed with other foods. The intended use of *Spirulina* microalgae is not similar to beet extract used to color lemonade a pink color.

(i) Name and Address of Notifier and Authorized Agent:

The notifiers for this GRAS determination are:

Cyanotech Corporation
74-4460 Queen Kaahumanu Hwy., #102
Kailua-Kona, HI 96740
Tel: 808-326-1353
Contact: Gerald R. Cysewski, Ph.D.; CEO. Email: grc@kona.net

Earthrise Nutritionals, Inc.
424 Payran Street
Petaluma, CA 94952
Tel: (760) 348-5027 (ext 21)
Contact: Amha Belay, Ph.D. Senior Vice President. Email: abelay@cts.com

The authorized agent for this GRAS notification is:

R. Todd Lorenz, Ph.D.
11034 West Ocean Air Drive, #252
San Diego, CA 92130
Tel: 858-481-7841; cell: 858-342-3874; email: tlorenz@mindspring.com

(ii) Common or Usual Name of the Notified Substance:

"Spirulina microalgae" is the common name of the substance. Cyanotech Corporation has trademarks to the name "Spirulina Pacifica" and "Hawaiian Energizer" which are marketed as a human dietary supplements. *Spirulina* produced and marketed by Earthrise Nutritionals Inc. as dietary supplements have various trademarks including Earthrise Spirulina, Spirulina Bio, Spirulina, Certified Organic and Spirulina Gold.

(iii) Applicable Conditions of Use:

Currently, *Spirulina* is manufactured into a powder or tablets and marketed as a dietary supplement. The intended GRAS use of the substance is as a source of protein and phytonutrients, such as carotenoids. *Spirulina* would be added to limited categories of food. The first category is that of the specialty granola/breakfast/power/energy bars and the second is powdered nutritional drink mixes such as "smoothies". Additionally, *Spirulina* will be added as a condiment to salads and pasta meals. The level of use for these foods would be from 0.5-3 grams of *Spirulina* per serving size. The population expected to consume these foods are those that are active, healthy and usually exercise on a regular basis. Vegetarians have long been typical and traditional consumers of *Spirulina*. The substance is not intended for infant formulas.

For each of these intended uses, *Spirulina* is not a color additive per 21 CFR 70.3 (f) as it is a food ingredient such as cherries, green or red peppers or chocolate that contributes it's own natural color when mixed with other foods. Furthermore, the intended use of *Spirulina* is similar to other approved GRAS substances such as Brown Algae (21 CFR 184.1120) and Red

Algae (21 CFR 184.1121). The intended use of *Spirulina* microalgae is not similar to beet extract used to color lemonade a pink color.

An estimated daily intake could be calculated in the following manner:

1) High-end Consumer: It could be estimated that a high-end user may consume 2 servings of food per day containing the highest levels of *Spirulina.* This may be one powdered smoothie beverage and one power bar containing 3 grams of *Spirulina* each for a total of 6 grams of *Spirulina* microalgae per day.

2) Medium Consumer: A medium user may consume 1 serving of food per day containing the highest level of *Spirulina.* This may be one powdered smoothie or power bar containing 3 grams of *Spirulina* microalgae per day.

3) Low-end Consumer: A low-end user may consume 1-4 servings per month resulting in an exposure of 3-12 grams of *Spirulina* microalgae per month if at the highest levels.

Spirulina has been tested in a number of foods and beverages at these levels and does not have a negative impact on organoleptic qualities (taste or smell) to these products. *Spirulina* does not degrade into any known carcinogenic or toxic substances.

(iv) Basis for GRAS Determination:

The basis the GRAS determination of *Spirulina* microalgae is by scientific procedures. Cyanotech Corporation and Earthrise Nutritionals, Inc. equally accept responsibility for this GRAS determination.

Data and information that are the basis for the notifier's GRAS determination are available for FDA review and copying at reasonable times at Cyanotech Corporation (Kailua-Kona, HI) or Earthrise Nutritional, Inc. (Petaluma, CA). Alternatively, the information will be sent to FDA upon request.

(2) Detailed Identity of the Notified Substance:

(i) Formal name:

Spirulina platensis, is also known as *Arthrospira.* It is classified in The Bergey's

Manual of Determinative Bacteriology (Ninth Edition) as the following:

Group 11	Oxygenic Phototropic Bacteria
Family	Cyanobacteria
Order	Oscillatoriales
Genus	Spirulina or Arthrospira
Species	platensis, maxima, fusiformis

Spiral shapes of the algae metamorphose spontaneously depending on pH and nutrient conditions. Thus, it is likely that different morphologies are simply variations of a single species ie *Spirulina platensis*, *Spirulina maxima*, *Spirulina fusiformis* (Dillon 1995). Recent molecular taxonomy studies also support this view (Scheldeman et al. 1999).

(ii) CAS Registry Number:

Not applicable, the substance is not a purified compound. ***Spirulina*** microalgae is blue-green microalgae produced under cGMP conditions. Additionally, Cyanotech Corporation and Earthrise Nutritionals Inc. produce the substance under an ISO-9002 and ISO-9001 registered Quality Systems, respectively. The final product is a free-flowing, dark blue-green powder with a mild seaweed smell, produced by spray drying whole the algae, *Spirulina platensis*. It consists of the usual proteins, carbohydrates, lipids, and minerals found in most plant products (Dillon, 1995).

(iii) Empirical and Structural formula, Molecular Weight, Formula Weight:

Not applicable, the substance is not a purified compound. ***Spirulina*** microalgae is blue-green microalgae produced under cGMP conditions. Additionally, Cyanotech Corporation and Earthrise Nutritionals, Inc. produce the substance under an ISO-9002 and ISO-9001 registered Quality System. The final product is a free-flowing, dark blue-green powder with a mild seaweed smell, produced by spray drying whole the algae, *Spirulina platensis*. It consists of the usual proteins, carbohydrates, lipids, and minerals found in most plant

products (Dillon, 1995).

(iv) Composition of and Identity of Mixture

(A) Physical Appearance and Identity

The substance can be readily identified as *Spirulina* under microscopic and biochemical examination. *Spirulina* powder is fine uniform powder, dark blue-green in color, with a mild seaweed taste and smell.

(B) General Composition *Spirulina* microalgae Powder

Component	Percentage
Proteins	60%
Carbohydrates	19%
Lipids	6%
Minerals	8%
Moisture	7%

(C) Typical Analysis of Minerals in *Spirulina*

Minerals	Amount (per 3 grams)
calcium	12.0 mg
magnesium	14.4 mg
iron	3.18 mg
phosphorus	31.2 mg
potassium	45.6 mg
sodium	21.9 mg
manganese	78 mcg
zinc	36 mcg
boron	30 mcg
copper	3 mcg
molybdenum	3 mcg

(D) Typical Analysis of Fatty Acids in *Spirulina*

Fatty Acids	Amount (per 3 grams)
Palmitic	60 mg
Myristic	1.24 mg
Stearic	0.204 mg
Arachidic	0.144 mg
Behenic	0.144 mg
Lignoceric	0.072 mg
Palmitoleic	5.94 mg
Oleic	0.51 mg
Erucic	0.072 mg
Gamma linolenic (GLA)	30 mg
Linoleic	33 mg
Dihomogamma linolenic	1.59 mg
Alpha linolenic	0.0435 mg
Docosahexenoic (DHA)	0.0435 mg

(E) Typical Analysis of Vitamins in *Spirulina*

Vitamins	Amount (per 3 grams)
Vitamin B1 (thiamin)	100 mcg
Vitamin B2 (riboflavin)	100 mcg
Vitamin B3 (niacin)	430 mcg
Vitamin B6	15 mcg
Vitamin B12 (cobalamin human-active)	1.47 mcg

(F) Typical Analysis of *Spirulina* Phytonutrients

Phytonutrients	Amount (per 3 grams)
cis beta-carotene	2.2 mg

Trans beta-carotene	7.8 mg
Zeaxanthin	0.95 mg
Chlorophyll a	30 mg
Phycocyanin	360 mg

Several glycolipids were isolated from *Spirulina* by systematic fractionation with different solvents. Structural investigation using methylation, GC-MS and enzymatic techniques indicated four major glycolipids. Main fatty acid components of these glycolipids were identified as palmitic acid and linoleic or linolenic acid. Based on this fatty acid composition, *Spirulina* glycolipids are comparable with those in higher plants (Kataoka, 1983: Dillion et al, 1995).

(v) Natural Origin, Geographical Factors and Information on Source:

Spirulina microalgae was "rediscovered" in the mid-1960's in a report by botanist, Jean Leonard, described a blue-green cake sold in the food market of Fort Lamy, Chad. A further study revealed the cake, locally called dihe', contained *Spirulina* and was consumed by the Kanembu tribe living along the alkaline lakes of Chad and Niger for many decades. Earlier reports in 1940 on the use of dihe' had passed without notice.

Soon thereafter, *Spirulina* was identified as growing in the sodium bicarbonate evaporation ponds at Sosa Texcoco near Mexico City. This lead to the first detailed and systematic study of the growth requirements and physiology of *Spirulina* by researchers. This study resulted in the Ph.D. thesis of Zarrouk and was the basis for the first large-scale production plant for *Spirulina*, ultimately located on Lake Texcoco. Ironically, Spanish invaders of Mexico in the 1500's discovered Aztecs gathering and selling blue-green *Spirulina* cakes called "tecuitlatl" in the local markets that was likely harvest from Lake Texcoco.

Spirulina is a widely distributed algae, found in alkaline and saline lakes of Africa, Asia, South and Central America (Busson, 1971; Clement 1971, 1975; Iltis, 1970, 1971, 1980; Guerin-Dumartrait 1976). *Spirulina* is a dried product from the common blue-green

microalgae, *Spirulina platensis*. The production strain was originally obtained from the University of Texas at Austin Algae Culture Collection. The *Spirulina* algae strain is non-GMO (genetically modified organism), non-toxigenic and non-pathogenic.

Spirulina has been marketed and consumed as a human food and has been approved as a food for human consumption by many governments, health agencies and associations of over 70 countries:

Argentina	Australia	Austria	Bahrain	Bahamas	Bangladesh
Belarus	Belgium	Brazil	Bulgaria	Canada	Chad
Chile	China	Colombia	Costa Rica	Croatia	Czech Republic
Denmark	Ecuador	Egypt	Ethiopia	Finland	France
Germany	Greece	Guam	Gulf States	Haiti	Hong Kong
Hungary	India	Iceland	Indonesia	Ireland	Israel
Italy	Jamaica	Japan	Kenya	Korea	Kuwait
Liechtenstein	Luxembourg	Macedonia	Malaysia	Mexico	Myanmar
Monaco	Netherlands	New Zealand	Nigeria	Norway	Peru
Philippines	Poland	Portugal	Romania	Russia	Saudi Arabia
Singapore	Slovenia	South Africa	Spain	Sweden	Switzerland
Taiwan	Thailand	Togo	Turkey	Ukraine	United Kingdom
United States	Venezuela	Vietnam	Yugoslavia	Zaire	Zimbabwe

Spirulina is commercially cultivated in numerous countries including the United States, China, India, Thailand, Taiwan, and Japan. The annual production is estimated to be over 3000 metric tons.

Currently, Cyanotech has 57 *Spirulina* ponds with an average size of 2,900 sq meters. The annual production capacity is approximately 400,000 kg per year. It is estimated that Cyanotech has produced a total of 3,046,400 kg (dry wt.) of *Spirulina* for human consumption since operations began in 1985. Earthrise Farms in California has 30 Spirulina ponds with an aveage size of 5,000 sq meters each. It has an annual production capacity of 500,000 kg and has

produced 3,000,000 kgs since its operation began in 1982. Currently, it is estimated that 250,000 kg *Spirulina* is imported from China and India into the US for human consumption

***Spirulina* production estimates are based on a world survey of researchers and producers (Metric Tons).**

Year	Burma	Chile	China	Cuba	India	Japan	Mexico	Taiwan	Thailand	Vietnam	USA-CA	USA-HI	Total
1975	0	0	0	0	0	0	20	0	0	0	0	0	20
1976	0	0	0	0	0	5	45	0	0	0	0	0	50
1977	0	0	0	0	0	11	65	4	0	0	0	0	80
1978	0	0	0	0	0	20	145	4	1	0	0	0	170
1979	0	0	0	0	0	20	200	9	50	0	1	0	280
1980	0	0	0	0	0	20	245	14	50	0	1	0	330
1981	0	0	0	0	0	30	250	19	50	0	1	0	350
1982	0	0	0	0	0	35	250	25	60	0	20	0	390
1983	0	0	0	0	0	45	250	25	60	0	50	0	430
1984	0	0	1	0	0	47	250	60	75	0	55	2	490
1985	0	0	1	0	0	53	250	60	100	1	55	10	530
1986	0	0	1	0	1	60	250	60	110	3	55	20	560
1987	0	0	3	0	3	60	250	60	110	4	70	40	600
1988	0	0	3	0	3	60	250	80	110	4	70	50	630
1989	0	0	8	0	6	50	250	80	110	6	70	60	640
1990	0	0	8	0	7	35	250	90	120	0	120	80	710
1991	4	1	8	0	7	20	250	90	120	0	160	100	760
1992	12	4	12	0	12	20	250	90	120	0	160	120	800
1993	15	5	20	2	20	20	225	90	120	3	160	120	800
1994	20	5	50	10	80	20	100	80	130	5	210	160	870
1995	25	7	120	20	150	20	0	50	150	8	370	250	1170
1996	30	20	250	40	250	20	0	60	150	10	480	400	1710
1997	30	50	500	50	250	20	0	70	150	20	500	450	2090
1998	30	70	700	60	300	20	100	80	150	20	600	500	2630
1999	30	90	900	70	500	20	100	80	150	20	800	600	3360

(vi) Isomeric properties:

The substance is not a purified compound. ***Spirulina*** is blue-green microalgae produced under cGMP conditions. Additionally, Cyanotech Corporation and Earthrise

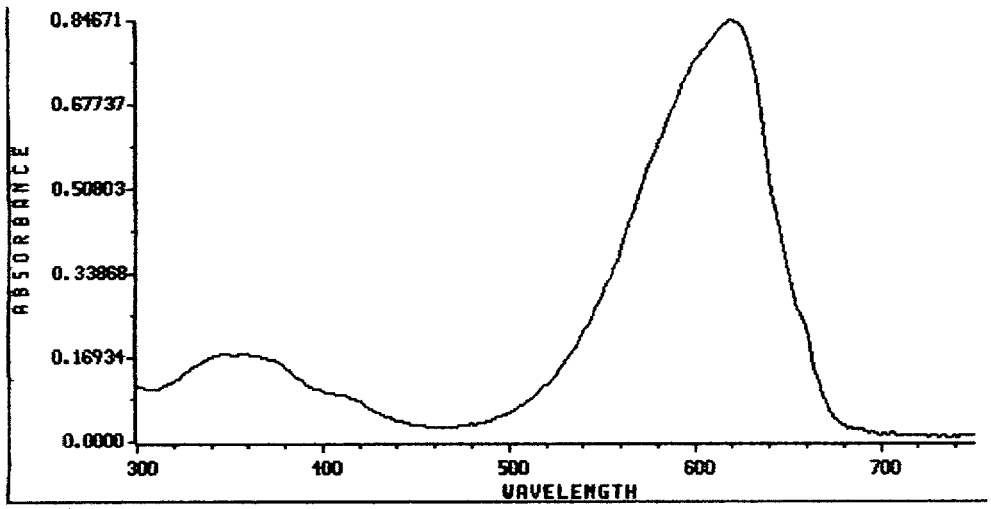
Nutritionals Inc. use an ISO-9002 and ISO-9001 registered Quality System. The final product is a free-flowing, dark blue-green powder with a mild seaweed smell, produced by spray drying whole the algae, *Spirulina platensis*. It consists of the usual proteins, carbohydrates, lipids, and minerals found in all plant products (Dillon, 1995).

(vii) Solubility and other physical properties

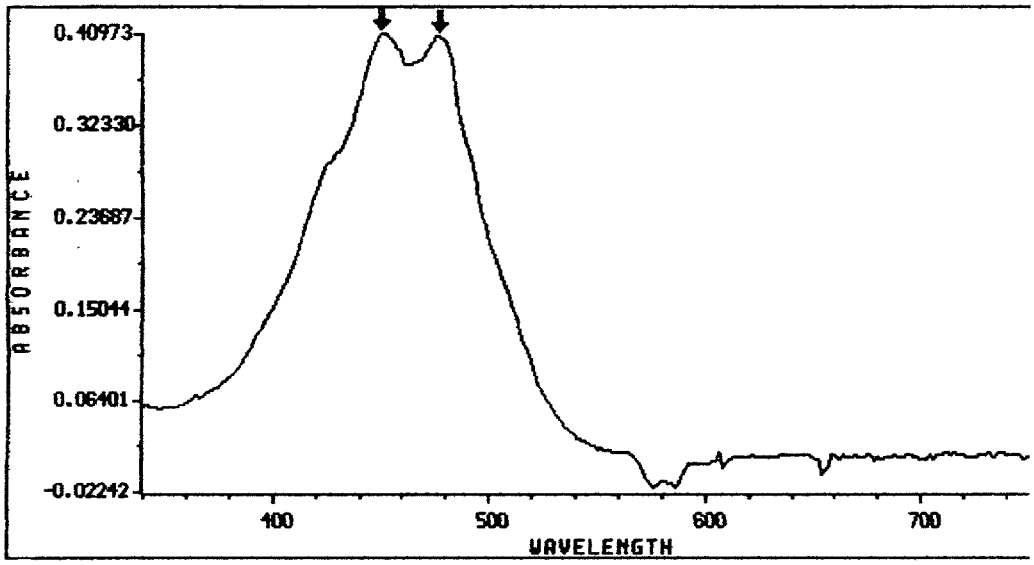
Spirulina microalgae is not readily soluble in water or solvents

(viii) Spectrophotometric properties of *Spirulina*

The phycocyanin from *Spirulina* has a characteristic peak at 620 nm as seen in the following spectrophotometric scan.



The major carotenoid extracted from *Spirulina*, beta-carotene, has a two characteristic peaks at 450 nm and 476 nm as seen from the following spectrophotometric scan.



(ix) Manufacturing Process

(A) Source and type of algae cultivated

The source strain is *Spirulina platensis* and was originally obtained from the University of Texas at Austin Algae Culture Collection. The production strain is non-GMO (genetically modified organism), non-toxigenic and non-pathogenic.

(B) Composition of growth medium

The growth media consists of water, sodium bicarbonate, sodium nitrate, sulfate potash, potassium, minerals and other typical plant nutrients. Nutrients are supplied by reliable manufacturers that include specifications for heavy metals and other possible contaminants.

No solvents, pesticides, herbicides or toxic substances are used during any cultivation or manufacturing step of the product. There are no carcinogens or compounds that are known in the scientific literature to degrade or metabolize to carcinogens, used in the

manufacturing process or known within *Spirulina*. Cyanotech Corporation and Earthrise Nutritionals, Inc. have part of their *Spirulina* production dedicated to organic production and are certified by Quality Assurance International (San Diego, CA).

(C) Manufacturing and Processing of *Spirulina*

Spirulina is grown in 30-64 separate ponds that are lined with reinforced polypropylene. Cultures are circulated by means of a paddlewheel. Pond nutrients are monitored and adjusted by laboratory chemists who conduct daily tests to assure consistency and optimal conditions. Ponds are harvested daily or from one to seven times per week by means of a pump and transferred into a dedicated processing building. The *Spirulina* culture is then subjected to a series of stainless-steel screens to rinse and concentrate the algae. The *Spirulina* slurry is then transferred by gravity to shaker screens for further concentration, and finally to a vacuum belt which accumulate the algae into a paste with a final washing step. The *Spirulina* paste is then pumped into a spray dryer to remove the moisture, resulting in a free flowing fine powder. The entire process from pond to powder takes less than 15 minutes.

Samples of the powder are taken in sterilized bags, labeled, and transferred to the Quality Control Laboratory for analysis of components and microbiological and quality assessment. The laboratory staff logs all data collected onto written sheets and into a database on the computer network, lots are graded to certain specifications according to customer requirements.

The dried powder is weighed and vacuum-sealed into various sized oxygen-barrier bags to minimize exposure to air and possible oxidation of nutrients such as beta-carotene and fatty acids. The bags are then packed into cardboard boxes or drums, sealed with tape and labeled to reflect the package weight and lot numbers for tracking purposes. Packaging materials do not transmit contaminants or objectionable substances to the product, and provide adequate protection from contamination. All operations in receiving, inspecting, transporting, packaging, segregating, preparing, processing, and storing of the product are conducted in accord with strict sanitation principles. All reasonable precautions are taken to

assure that production procedures do not contribute contamination such as filth, harmful chemicals, undesirable microorganisms, or any other objectionable material to the processed product.

(D) Facilities

Spirulina is presently cultivated and processed in two locations in the United States. The location of the facilities are:

73-4460 Queen Kaahumanu Highway, #102

Kailua-Kona, HI 96740

Telephone: 808-326-1353

FAX: 808-329-3597

Earthrise Nutritional, Inc.

113 East Hoober Road (PO Box 270)
Calipatria, CA 92233
Tel. 760-348-5027
Fax: 760-348-2895

Cyanotech Corporation has Food Establishment Permits issued by the State of Hawaii for both *Spirulina* production and *Haematococcus* algae and Earthrise Nutritionals has a registration with the State of California to manufacture, process and pack and hold food products.

(E) Process Controls

Spirulina powder is manufactured in accordance with Current Good Manufacturing Practices promulgated under the United States Federal Food, Drug and Cosmetic Act and applicable Hawaii and California statues and regulations. These laws assure that the facilities, methods, practices, and controls used in the manufacture, processing, packing, or holding of food products are in conformance with or are operated in conformity with Good Manufacturing Practices to assure that the food products are safe for consumption and has been prepared, packed, and held under sanitary conditions.

Raw materials and ingredients are inspected and segregated as necessary to assure that they are clean, wholesome, and fit for processing and are stored under conditions that will protect against contamination and minimize deterioration. Packaging materials do not transmit contaminants or objectionable substances to the product, and provide adequate protection from contamination.

All operations in receiving, inspecting, transporting, packaging, segregating, preparing, processing, and storing of the product are conducted in accord with adequate sanitation principles. All reasonable precautions are taken to assure that production procedures do not contribute contamination such as filth, harmful chemicals, undesirable microorganisms, or any other objectionable material to the processed product.

The Quality Control Department and GMP Coordinator have the responsibility and authority to approve or reject all raw materials, in-process materials, packaging materials, final product and labeling, and the authority to review production records to assure that no errors have occurred or, if errors have occurred, that they have been fully investigated. The Quality Control Department has the responsibility for approving or rejecting all procedures or specifications impacting on the identity, strength, quality, and purity of the final product. Adequate laboratory facilities for the testing and approval (or rejection) of raw materials, in-process materials, packaging materials and final product are available to the Quality Control Department.

The manufacturing facility is audited and inspected for violations of Good Manufacturing Practices on a regular basis by the GMP Coordinator. Cyanotech Corporation and Earthrise Nutritionals, Inc. have over 25 years of manufacturing experience with *Spirulina* algae sold into the health food market. The companies have periodic unannounced inspections from State and local representatives, and have never been cited for a major violation or issued a Form 483 violation of Good Manufacturing Practices. On October 7, 1996 Cyanotech Corporation was issued a Certificate of Registration from Orion Registrar Inc. for ISO 9002-94 Quality Management System (Certificate Identification HI-1-96-1 EAC/SIC Code: 13/2833). Earthrise obtained ISO-9001 registration on July 31, 1998 from Perry Johnson Registrars, Inc. Certificate Number 98-242. The ISO certificates are both currently in good standing. Earthrise Nutritionals Inc. has also attained a NNFA Dietary Supplement Good Manufacturing Practices Certification

in May 2001.

Woodson-Tenent Laboratories, Inc. (FDA Registration 1020132, Memphis, TN) or Covance Laboratories Inc. periodically conduct testing of finished products, including proximate analyses, pesticides, heavy metals, and other toxic contaminants.

(x) Specifications for Food-Grade Material

(A) Description:

Spirulina is a free-flowing, dark blue-green powder with a mild seaweed smell, produced by spray drying whole the algae, *Spirulina platensis*. It consists of the usual proteins, carbohydrates, lipids, and minerals found in all plant products Dillon, 1995).

(B) Identification Tests:

The substance can be readily identified as *Spirulina* by microscopic and biochemical examination. *Spirulina* powder is fine uniform powder, dark blue-green in color, with a mild seaweed taste and smell.

(C) Assay for Substance

There is not a single assay for the substance as it is not a pure substance and may be considered a whole plant. However, certain components of the substance, such as carotenoids, chlorophyll-a, or phycocyanin, can be assayed. The methods used by the two companies may vary slightly.

(1) HPLC Analysis of Spirulina Carotenoids and Spectrophotometric Analysis of Total Carotenoids

Part 1 Extraction in Methanol
Materials
Metric balance
Hach tubes (12 ml) with caps
Glass beads
Water bath (45 degree C.)
Vortexer
Centrifuge

Volumetric flask (25 ml)
DMSO
Methanol (Reagent Grade)
Graduated pipettes
Pasteur pipettes and rubber balls

Procedure

1) Perform the analysis in duplicate for each sample.
2) Weigh approximately 30 mg of Spirulina powder directly into Hach Tubes. Record the weight.
3) Add 3 grams of glass beads and 2.5 ml DMSO to each tube.
4) Tightly cap the tubes and vortex them briefly for 30 seconds.
5) Place the tubes into a 45 degree water bath for 20 minutes. Every 10 minutes remove tubes from the water bath and vortex them for 30 seconds.
6) After 20 minutes in the water bath, remove the tubes.
7) Add 5 ml of methanol to each tube, cap the tubes and vortex them vigorously for 30 seconds. Centrifuge the tubes at 4200 rpm for 3 minutes.
8) With a Pasteur pipette, pipette the supernatant from each tube into a volumetric flask.
9) Repeat step 7 and 8 until the centrifuged methanol is colorless. Three extractions should be ample.
10) After all the supernatant is collected in the volumetric flask, bring the flask up to volume with methanol.
11) Place the stopper in the volumetric flask and invert gently to mix the contents.

Part 2 Saponification and Preparation for HPLC

Materials

Methanol Extract obtained in Part 1
Saturated potassium chloride in water
25 % di-ethyl ether in hexane (reagent grade)
DI water
Hach tubes
vortexer
centrifuge
Sodium sulfate anhydrous (NaSO4)
Nitrogen manifold
Pasteur pipettes
3 ml volumetric pipette
1 ml calibrated pipette
3 ml volumetric flask
Running solvent (18 % acetone in hexane - HPLC grade)

Procedure

1) With the 3 ml volumetric pipette remove 3 ml of extract from the 25 ml volumetric flask and put it into a clean Hach tube.
2) Add 3 ml of 25 % diethyl ether in hexane to the tube.
3) Add 0.5 ml saturated KOH in water to the tube.
4) Cap the tube and vortex lightly to mix.
5) Place tube in a dark place for 30 minutes.
6) Remove the cap from the tube and add exactly 1 ml of DI water.
7) Cap the tube and vortex briefly to mix.
8) Centrifuge the tube at 4200 rpm for 3 minutes.
9) Add 1 gram of NaSO4- anhydrous to a clean tube - one for each sample.
10) Remove the ether-hexane layer from the centrifuge tube with a Pasteur pipette and put it into the Hach tube the NaSO4- anhydrous. Use additional aliquots (approximately 1 ml) of the 25 % ether in hexane to totally rinse out any color that remains in the top of the centrifuged tube where the solvent layer was. Combine all the solvent into the Hach tube with the NaSO4.
11) With the Pasteur pipette gently draw in and expel the solvent to expose the solvent to the NaSO4 to remove any water.
12) Transfer the solvent to a clean Hach tube, rinsing the NaSO4 with small aliquots of 25 % ether in hexane.
13) Evaporate the ether-hexane solvent from the Hach tube with a gentle stream of gas from the nitrogen manifold.
14) When the tube is completely dry add a small amount of running solvent to the tube and pipette the liquid into a 3 ml volumetric flask. Rinse the tube with the addition of small aliquots of running solvent and transfer it to the 3 ml volumetric flask. Bring the flask up to volume.
15) Transfer the contents of the volumetric to a clean Hach tube for the HPLC analysis.

Part 3 HPLC Analysis

Materials
Luna column with Safety Guard pre-column
Solvent system, hexane:acetone (86:14)
Standard Operating Procedure for Beckman HPLC
Percent Dry Weight Calculation

Calculations:

Consult the SOP for the Beckman HPLC for detailed operating directions using method "sp2". After the run the calculation for sample amount is:

Sample Amount = (methanol (ml) / (sample wt (mg) X Percent dry wt (decimal))/10

E.g. Sample Amount =((25 ml) / (30 mg X .95))/10 = .0877192

Part 4 Spectrophotometric Analyses for Total Carotenoids

Materials

Methanol extract obtained in Part 1
2 ml pipette
3 ml volumetric pipette
15 ml conical glass centrifuge tubes, calibrated "to contain" with 0.1 ml graduations and screw top lids.
Saturated KOH in water
Diethyl ether
DI water
Vortex
Scanning Spectrophotometer
Pasteur pipettes
Dry weight of the Spirulina sample

Procedure

1) Analyze each sample in duplicate.
2) Pipette exactly 2 ml of the methanol extract in to a 15 ml centrifuge tube.
3) Add exactly 3 ml of diethyl ether with a volumetric pipette to the Hach tube.
4) Add 0.33 of saturated KOH to the tube for a short saponification. Cap the tube and vortex briefly to mix. Set the samples in a dark place for 15 – 30 minutes for saponification.
5) Remove the cap and add 5 ml of water to each sample.
6) Replace the cap and vortex the tube vigorously for 30 seconds. Centrifuge the tubes for 3 minutes at 4200 rpm.
7) The ether layer should contain all the yellow pigments and the aqueous layer should be a pale blue-green.
8) Remove the cap from the centrifuge tube and add diethyl ether to the ether layer until it is exactly at a 3 ml volume according to the increments on the tube.
9) The absorbance of the ether layer is read on the spectrophotometer. Read the maximum absorbance (approximately 450-453) against a diethyl ether blank.

Calculation

$$\text{Total Carotenoids (percent)} = \frac{\text{Max Abs (450-453)}}{259.2 \times \text{sample wt (mg)} \times \text{dry wt}} \times 25 \text{ ml} \times 1.5 \times 100$$

(2) Quantitative Analysis of C-Phycocyanin from *Spirulina*

Background:

Spirulina and other blue-green algae contain c-phycocyanin, which acts as an accessory pigment when light energy is captured and transferred to chlorophyll a. This is a spectrophotometric method adapted to extract and quantify a relatively pure c-phycocyanin fraction from Spirulina.

Equipment and instruments:

Spectrophotometer at 620nm
Refrigerator (4 C)
Phosphate buffer (pH 7.0)*
10 ml centrifuge tubes
Cooled centrifuge (10 C @ 3500 RPM)
Desiccator
Weigh pans
Analytical balance

Dry Weight

1) Place drying pans in oven for 30 minutes place in desiccator to remove excess moisture.
2) When pans are cool, weigh and record weight of pan.
3) Tare the balance with the pan on it and place about two grams of powder in the pan.
4) Record the weight of the powder.
5) Place pan and powder in the oven to dry for six hours.
6) Remove pan and powder from the oven and place in desiccator 15 minutes to cool.
7) Weigh and record the total weight of the pan and the dry powder.
8) Perform duplicates for each sample.

Dry Weight Calculations

$$\text{Percent dry wt} = \frac{(\text{pan (g)} + \text{dried powder (g)}) - \text{pan wt (g)}}{\text{powder wt (not dried) (g)}}$$

Phycocyanin Assay

1) Perform analysis in duplicate.
2) Weigh accurately 30 mg. Spirulina powder into a 10-ml centrifuge tube.
3) Add 10 mls. of the 100 mM phosphate buffer (100-mM Phosphate buffer contains 10.64 g. K_2HPO_4 and 5.29g. KH_2PO_4 per liter, pH 7.).
4) Vortex to mix well.
5) Store in refrigerator overnight.
6) Vortex to mix well.
7) Centrifuge 5 minutes at 10 C at 3500 RPM.

8) Read absorbency of each replicate at 620 nm, using phosphate buffer as blank.
9) Average absorbency readings for dilution replicates.

Derivation of pure C-Phycocyanin:

$$\% \text{ pure CPC} = \frac{A_{620} \times (10) \times (100)}{7.3 \times (\text{mg. sample}). \times (\% \text{ dry wt.})}$$

where 7.3 is Extinction coefficient of CPC at 620 nm
10 is total volume;
100 represents 100%.

Derivation of crude C-Phycocyanin:

$$\% \text{ crude CPC} = \frac{A_{620} \times (10) \times (100)}{3.39 \times (\text{mg. sample}). \times (\% \text{ dry wt.})}$$

where 3.39 is Extinction coefficient of CPC at 620 nm
10 is total volume;
100 represents 100%.

Reference:

Boussiba S. and A. Richmond. 1979. Isolation and Purification of Phycocyanins from the Blue-Green Alga *Spirulina platensis*. Arch. Microbiol. 120:155-159.

(3) Quantitative Analysis of Chlorophyll-a from *Spirulina*

Background:

Spirulina contains only Chlorophyll-a. Carotenoids and chlorophyll are acetone soluble but the carotenoids do not interfere in this spectrophotometric method as the absorbency for carotenoids range from 400-500 nm, and the absorbency for chlorophyll A is 666 nm.

The other major pigments in *Spirulina* are the water-soluble phycocyanin. These remain in an acetone-insoluble pellet during the assay.

Equipment and instruments:

Analytical balance
Centrifuge
Drying pans
Drying oven at 110 degrees C.

85 % acetone in water
Spectrophotometer
35 ml round bottom glass centrifuge tubes with caps
50 ml volumetric flask with lid
Vortexer (Maxi Mix II)
Centrifuge
Glass beads (through 20 mesh)
Pipettes

Dry Weight

1) Place drying pans in oven for 30 minutes place in desiccator to remove excess moisture.
2) When pans are cool, weigh and record weight of pan.
3) Tare the balance with the pan on it and place about two grams of powder in the pan. Record the weight of the powder.
4) Place pan and powder in the oven and dry for two hours.
5) Remove pan and powder from the oven and place in desiccator 15 minutes to cool.
6) Weigh and record the total weight of the pan and the dry powder.
7) Perform duplicates for each sample.

Chlorophyll A Assay

1) Weigh approximately 50 mg of Spirulina into a 35 ml centrifuge tube. Record weight.
2) Add 5 grams of glass beads and 2.5 ml of 85 % acetone in water.
3) Vortex vigorously for 5 minutes.
4) Add 10 ml of 85% acetone in water, vortex briefly and centrifuge at 3200 RPM of 5 minutes.
5) Collect the supernatant in a 50 ml volumetric flask .
6) Repeat steps 3-5 until supernatant is clear. Four extractions should be sufficient.
7) Bring the flask up to volume with 85% acetone in water and cap the flask and invert gently to mix the contents.
8) Read the absorbency with the spectrophotometer at 666nm and 642 nm against an 85 % acetone/water blank.

Calculations:

Dry weight

$$\text{Percent dry wt} = \frac{(\text{pan (g)} + \text{dried powder (g)}) - \text{pan wt (g)}}{\text{powder wt (not dried) (g)}}$$

Chlorophyll A

$$\text{Chlorophyll A (\%)} = \frac{[(9.93 \times Abs_{666}) - (0.0777 \times Abs_{642})] \times 0.05 \text{ liter} \times 100}{\text{Sample weight (mg)} \times \text{\% dry wt.}}$$

References

A.O.A.C. Official Methods of Analysis (1995); 940.03

(xi) Physico-chemical Characteristics, Impurities and Contaminates of the Substance.

Quality and Safety Standards for Spirulina
for the USA Natural Foods Industry

The Natural Products Quality Assurance Alliance (NPQAA)
and The Natural Nutritional Foods Association (NNFA)

Extraneous Materials.

For USA human consumption only, testing of each production lot is required. *US FDA Guideline acceptance criteria.

1. Insect fragments	*less than 150/50g	AOAC (1990) 15th ed.
2. Rodent hairs	*1.0/100g	AOAC 990.09

Heavy Metals. Shown by a typical analysis of spirulina:

1. Lead	less than 2.5 ppm	AOAC
2. Arsenic	less than 1.0 ppm	AOAC
3. Cadmium	less than 0.5 ppm	AOAC
4. Mercury	less than 0.05 ppm	AOAC

Supplementary Guidelines. Shown by a typical analysis of spirulina:

1. No pesticides	4.	No preservatives
2. No herbicides	5.	No stabilizers
3. No dyes	6.	No irradiation

Spirulina finished products

Finished products for human consumption shall meet all relevant USA food quality and safety standards, and shall follow the appropriate Good Manufacturing Practice Guidelines.

Minimum Nutritional Content. To be determined.

Moisture. Acceptance criteria for each production lot:

6. Moisture	less than 7%	AOAC

Bacteriological Assays. Acceptance Criteria:

1. Standard Plate Count	less than 200,000/g	FDA Bacteriological Manual
2. Molds	less than 100/g	FDA Bacteriological Manual
3. Yeast	less than 40/g	FDA Bacteriological Manual
4. Coliforms	less than 3/g	FDA Bacteriological Manual
5. Salmonella	negative	FDA Bacteriological Manual
6. Staphylococcus	negative	FDA Bacteriological Manual

Product shelf life:

Producers of finished products shall determine nutrient statements on labels based on both bulk spirulina powder analysis and nutrient changes due to tableting and bottling and package shelf life.

(xii) Other Possible Tested Contaminants of *Spirulina*

Spirulina algae is non-GMO and grown without pesticides, herbicides, antibiotics or hormones. Periodic analysis of contaminates is conducted by Woodson-Tenent Laboratories or Covance Laboratories.

Contaminate	**Concentration**
hexachlorobenzene-HCB	<0.01 ppm
BHC	<0.01 ppm
lindane	<0.01 ppm
heptachlor	<0.01 ppm
aldrin	<0.01 ppm
heptachlor epoxide	<0.01 ppm
DDE	<0.01 ppm
dieldrin	<0.01 ppm
endrin	<0.01 ppm
DDD	<0.01 ppm
DDT	<0.01 ppm
mirex	<0.01 ppm
methoxychlor	<0.01 ppm
chlordane	<0.01 ppm
toxaphene	<0.20 ppm
PCB total	<0.10 ppm
diazinon	<0.01 ppm
methyl parathion	<0.01 ppm
malathion	<0.01 ppm
ethyl parathion	<0.01 ppm
ethion	<0.01 ppm
ronnel	<0.01 ppm

(xiii) Stability of the Added Substance

Carotenoids of *Spirulina* are the most sensitive component and can degrade due to heat, light and oxygen. Precautions are taken during manufacturing and processing to limit these conditions as much as possible. Dried *Spirulina* is packed into airtight bags and then vacuum-sealed before shipping for extraction.

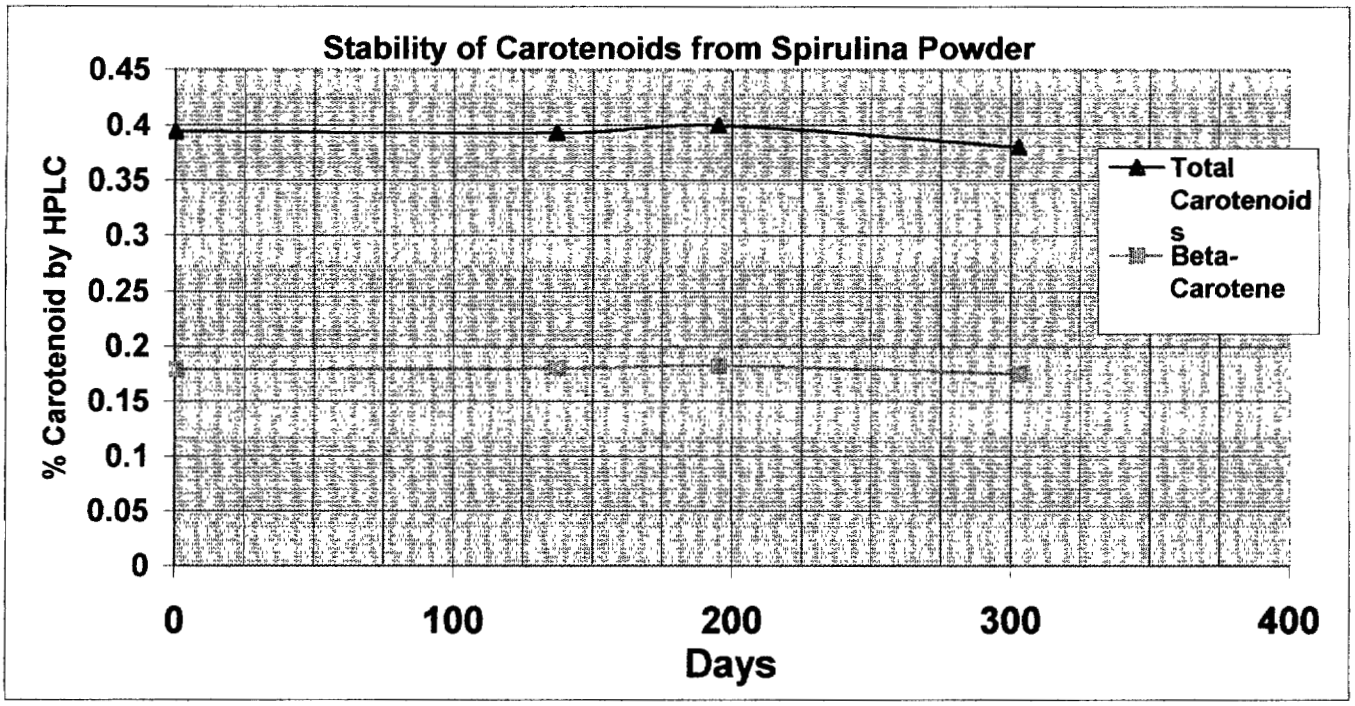
Labeling and instruction of the substance includes pertinent information about shelf life and the best storage and handling conditions for optimal stability.

Stability Study

Product Name: *Spirulina*
Shelf life: 3 years

	Specification	Initial	1 yr.	2 yr.	3 yr.	4 yr.	5 yr.
Appearance:	dark blue-green	same	same	same	same	same	same
Consistency	powder	same	same	same	same	same	same
Smell	mild seaweed smell	same	same	same	same	same	same
Taste	mild seaweed taste	same	same	same	same	same	same
phycocyanin	8.6%	8.6%	8.6%	8.2%	8.0%	7.5%	7.0%
carotenoids	450 mg%	450	450	425	400	350	300
E. coli.	neg.	neg.	neg.	neg.	neg.	neg.	neg.
Salmonella	neg.	neg.	neg.	neg.	neg.	neg.	neg.

A second stability study of the carotenoids (total carotenoids and beta-carotene) from *Spirulina* packed in vacuum-sealed bottles is shown that demonstrates very little loss after more than 300 days.



(3) Self-limiting levels:

There are no self-limiting levels for *Spirulina* microalgae. The substance is currently marketed as a dietary supplements in the form of tablets and powder. The taste and smell of the substance is similar to mild seaweed.

(4) Detailed summary of GRAS determination:

It has been recommended by experts to eat 5 servings of fruits and vegetables per day. Additionally, mixed natural carotenoids should be used as supplements in the human diet for those that do not eat the proper diet. The typical doses of carotenoids are 20-60 mg (33,000-100,000 IU) per day for beta-carotene, 10-30 mg of alpha-carotene, and 3-6 mg each of lutein, lycopene and zeaxanthin (Yarnell). *Spirulina* is a source of beta-carotene (0.2% dry wt.) and zeaxanthin (0.1% dry wt.). These carotenoids are commonly found in fruits and vegetables of the normal human diet.

Spirulina has a long history and documented record of human consumption, and is known in the literature and by prominent researchers as a safe and nutritious microalgae. In the past 20 years, it has been marketed and consumed as a safe human food by millions of people in

North and South America, Asia, Europe and Africa. Furthermore, *Spirulina* has been approved as a food for human consumption by many governments, health agencies and associations of over 60 countries. Based on 30 years of safety and quality research, many countries and organizations have established *Spirulina* quality and safety standards, which are abided to by Cyanotech Corporation and Earthrise Nutritional, Inc. *Spirulina* can be cultivated under scientifically controlled conditions that virtually eliminate contamination by other algae.

Numerous data published in the primary published scientific literature, including work with malnourished children, does not raise any safety issues with *Spirulina* and thereby provides abundant evidence that there is a consensus among qualified experts, including the United Nations Food and Agriculture Organization (UNFAO) and United Nations Industrial Development Organization (UNIDO), that there is reasonable certainty that the substance is not harmful under the intended conditions of use.

Considering the totality of other corroborative evidence, the safety studies with animals and humans demonstrate there is a reasonable certainty that *Spirulina* is not harmful under the intended conditions of use. Toxicity studies in both animals and humans demonstrate no adverse effects.

(i) Animal Safety Studies

In the 1970s, *Spirulina* underwent extensive safety studies with animals and fish. Independent feeding tests in France, Mexico and Japan showed no undesirable results and no toxic side effects on humans, rats, pigs, chickens, fish and oysters. Many independent rat-feeding trials were conducted in Japan and no negative effects were found for acute, chronic toxicity or reproduction (Takemoto, K., 1982; Atatsuka, K., 1979). Long-term feeding studies with rats have shown no toxicity or adverse effects (Boudene, 1976). Further published studies from independent laboratories confirm the absence of any toxic or mutagenic effects when *Spirulina* provides a significant portion of the dietary protein (Til H.P, 1971; Fevier C., 1976; Ciferri O., 1983).

In 1980, one of the most comprehensive *Spirulina* animal studies was sponsored by the United Nations Industrial Development Organization (UNIDO) using rats and mice. In this study, *Spirulina* was incorporated into the food at 10-35% of the total diet. There were no problems with second or third generation reproduction, fertility, lactation or birth defects found. No cancer causing properties were found. No problems with heavy metals, nucleic acids, pesticides or bacteria were found (Chamorro-Cevallos, G., 1980).

Toxicology research has continued through the 1980s and 1990s, showing *Spirulina* has no peri- or postnatal toxicity in rats, no adverse effects on reproduction, including male and female fertility, duration of gestation, and no increase in number of abnormal offspring, or effects on uterus or ovaries (Becker, W. E., 1984; Chamorro, G, 1988 & 1997; Salazar, M., 1989, 1996, 1998). In another study, *Spirulina* was incorporated into experimental diets at levels of 10-30% and fed to groups of adult CD-1 mice. Five day short-term and prolonged-term (5 d/week for 10 weeks) feeding was followed by mating with untreated virgin females. Examination of uteri and ovaries of pregnant females on day 12-14 of gestation for counting preimplantation losses and non-living implants failed to reveal dominant lethal effects (Chamorro, 1989). Pregnant rats were fed five different diets providing 22% protein to study the supplementary effects of *Spirulina*. Rats receiving *Spirulina* produced significantly higher litter sizes than those with casein and wheat gluten. Birth weights of the pups from the *Spirulina* group were comparable to the control group. The authors concluded that *Spirulina* appears to be a good dietary supplement during pregnancy (Kapoor, 1993).

Kidney toxicity caused by mercury was suppressed by feeding *Spirulina* to rats. A water-soluble extract of *Spirulina*, containing phycocyanin, the natural blue pigment, was examined. Renal toxicity in rats caused by p-aminophenol and cisplatin was significantly reduced by the phycocyanin extract of *Spirulina*. Researchers concluded that phycocyanin plays a major role in the protective effect of *Spirulina* against renal failure caused by mercury and pharmaceutical drugs, and that *Spirulina* might be applicable to the reduction of general renal dysfunction (Fukino, 1990).

A chronic intoxication test with *Spirulina* was conducted on Wistar rats of both sexes for six

months. Rats were fed ad libitum either a 20% *Spirulina* diet or a control. The weight and appearance of organs were observed with regard to the brain, heart, stomach, liver, spleen, kidneys, testes or ovaries, and adrenal glands. There were insignificant differences between the experimental and control groups in the growth, external appearance, shape, weight and histological findings of the organs. Hematological tests showed some statistical differences e.g. Hb and SGPT of the male and total bilirubin of the female, though abnormal findings were not detected. The authors speculated that the *Spirulina* diet does not cause any toxic effects within 6 months of feeding (Yoshino, 1980).

Young rats reared for 15 weeks on a diet of 5% *Spirulina* grew favorably with good food efficiency and protein. The amount of fat accumulated was small as well as fat synthesis from carbohydrates compared to the control group. A reduction in the amount of fat in the liver was observed (Watanabe, 1986).

Two experiments with weaned pigs were conducted to evaluate the feasibility of partially replacing soy protein in a basal corn-soybean meal with proteins from *Spirulina*. There were no signs of diarrhea, loss of appetite, toxicity, or of gross histopathological lesions of the gastrointestinal tract, kidney, liver, or femur in pigs fed any of the diets. Blood hemoglobin and serum protein, albumin and urea concentrations were similar in all groups of the experiments. It was suggested that at least one-half of the protein supplied by the soybean meal, can be replaced in the diet of the early weaned pig by *Spirulina* without adverse effects (Yap, 1982).

(ii) Prior Use in the Human Diet & Human Safety Studies

Spirulina has an ancient history as a human food, and was regularly consumed by the Aztecs in North America prior to the Spanish conquest in the 1500's. The Aztecs harvested *Spirulina* from the surfaces of Texcoco and Tutalcingo lakes with fine-meshed nets and dried the paste under the sun. *Spirulina* was made into cakes 3-4 centimeters thick and which was called "techuitlatl". These cakes were made into breads, grain dishes and sauces called 'chilmolli'. *Spirulina* techuitlatl cakes were regularly traded as a commodity. There is also evidence that the Mayas of Central America (300-900 AD) cultivated *Spirulina* for foods in a system of waterways, which was also used for crop irrigation (Furst, 1978).

Spirulina has also been consumed for centuries as a major source of protein by the Kanembu people who live along the shores of Lake Chad in Africa. *Spirulina* is collected from the waters edge in fine-woven baskets, transferred to clay pots or gourds, and dried under the sun into small biscuits called "dihe' ". Dihe' is combined into the majority of sauces and is eaten in up to 70% of their meals, amounting to about 10-12 grams per person per meal. In times of famine, dihe' is a main ingredient of their diets (Abdulquader et. al, 2000; Furst, 1978; Ciferri, O., 1983; Delpeuch, 1976; Dillon, 1995).

In 1970, the attention of the United Nations Food and Agriculture Organization (UNFAO) was attracted by the fact that humans were consuming algae. The UNFAO organized an educational campaign in Chad to encourage consumption of *Spirulina* harvested from natural sources. More than 6000 meals were distributed under the supervision of the FAO and the campaign was considered a success (Institut Francais du Petrol. Rapport ou Comite Consultatif des Proteines OAA/ OMS/ FISE- Etat d'Avancement du Procede IFP de Production d'algues Dec 1970, p. 11). Another report stated "dihé" (*Spirulina* sauce) was served at the school canteen where the majority were the Kanembou people. The consumption of *Spirulina* in the children's food caused no problems of illness. *Spirulina* was consumed by non-Kanembou people at Fort Lamy, now called Ndjemena (Fadoul, L., 1971).

Spirulina was given to malnourished children and adults in clinical studies beginning in the early 1970s. Since the late 1970s, millions of people in developed countries have used it as a health food supplement, taking 3 to 20 grams a day. Rarely are there any reports of allergies or sensitivities.

A one year feeding program with 5,000 pre-school children showed a symptom of Vitamin A deficiency, "Bitot's spot", decreased from 80% to 10%. These rural children near Madras consumed 1 gram of *Spirulina* a day for at least 150 days. This small amount provided the daily requirement of beta-carotene (Vitamin A), which can help prevent blindness and eye diseases. In another study with 400 school children, a daily dose of beta-carotene from *Spirulina* increased their Vitamin A status to the same level as those administered pure Vitamin A. *Spirulina* was

given to children in a unique way: extruded noodles, sweetened with sugar to preserve the beta-carotene. Called "Spiru-Om", it was well accepted by the children. The project was sponsored by the Indian Government (Seshadri. 1993. Large scale nutritional supplementation with *Spirulina* alga. All India Coordinated Project on Spirulina. Shri Amm Murugappa Chettiar Research Center (MCRC) Madras, India).

Spirulina tablets were given to 21 patients with various nutritional deficiencies. They had suffered weight loss in conjunction with gastric resection, tubercular infection, chronic pancreatitis and gastritis, rheumatoid arthritis, anemia and diabetes mellitus. With *Spirulina,* the patients gained weight and their protein profiles improved (Fica, 1984).

In Nanjing Childrens Hospital, 27 children, 2-6 years old, recovered in a short period from bad appetite, night sweats, diarrhea and constipation from a baby nourishing formula containing 1.5g of *Spirulina*, 12g baked barley sprout, Vitaimn B1 and zinc. The clinical effects showed *Spirulina* was safe and healthy for children (Ren, 1987).

Spirulina with high zinc content may be twice as effective as a zinc supplement in curing zinc deficiency in children. The effective dose of zinc from *Spirulina* was 2 to 4 times less than the zinc from a common supplement, zinc sulfate. More than two times the children were cured with high zinc *Spirulina.* One hundred children were diagnosed as suffering from zinc deficiency. For a three-month period, 50 children were given zinc sulfate and 50 were given *Spirulina* tablets. Doctors concluded that *Spirulina*'s effect was much better than zinc sulfate. *Spirulina* had no side effects and was easy to administer for long periods of time. They theorized that high zinc *Spirulina* had many bioactive and nutritious substances, which improved mineral absorption, general health and the immune system (Yonghuang, 1994).

Spirulina has about 4% nucleic acids (DNA and RNA), lower than *Chlorella* and other microalgae, yeast and fungi (6-11%). One study found that uric acid levels did not increase in humans taking up to 30 grams a day of *Chlorella* protein or 50 grams of *Chlorella* (Waslein, 1970). Since *Spirulina* is lower in nucleic acid content, eating up to 50 grams a day can be safely used as major protein source (Jassby, 1988).

Thirty healthy men with high cholesterol, mild hypertension and hyperlipidemia showed lower serum cholesterol, triglyceride and LDL levels after eating *Spirulina* for eight weeks. These men did not change their diet, except adding *Spirulina*. No adverse effects were noted. Group A consumed 4.2 grams daily for eight weeks. Total serum cholesterol dropped a significant 4.5% within 4 weeks from 244 to 233. Group B consumed *Spirulina* for four weeks, then stopped. Serum cholesterol levels decreased, then returned to the initial level. Researchers concluded *Spirulina* did lower serum cholesterol and was likely to have a favorable effect on alleviating heart disease since the arteriosclerosis index improved (Nakaya, 1988).

The bioavailability of total carotenes and beta-carotene from *Spirulina* was examined in apparently healthy preschool children and found to be comparable to those values reported for other plant sources like leafy vegetables and carrots. The study also showed *Spirulina* is a good source of vitamin A, as there was a significant increase in serum retinol levels. Researchers concluded *Spirulina* can be used as a source of Vitamin A in the diet, is relatively inexpensive, has higher beta-carotene than any other plant source that can be cultivated throughout the year (Annapurna, 1991).

The blue-green microalgae *Spirulina*, used in daily diets of natives in Africa and America, has been found to be a rich natural source of proteins, carotenoids and other micronutrients (Dillon, 1995). Experimental studies in animal models have demonstrated an inhibitory effect of *Spirulina* algae on oral carcinogenesis. Studies among preschool children in India have demonstrated *Spirulina* to be a effective source of dietary vitamin A. The chemopreventative activity of *Spirulina* (1 g/day for 12 months) was evaluated in reversing oral leukoplakia in pan tobacco chewers in Kerala, India. Complete regression of lesions was observed in 20 of 44 (45%) evaluable subjects supplemented with *Spirulina*, as opposed to 3 of 43 (7%) in the placebo arm. When stratified by leukoplakia, the response was more pronounced in homogeneous lesions: complete regression was seen in 16 of 28 (57%) subjects with homogeneous leukoplakia, 2 of 8 with erythroplakia, 2 of 4 with verrucous leukoplakia, and 0 of 4 with ulcerated and nodular lesions. Within one year of discontinuing supplements, 9 of 20 (45%) complete responders with *Spirulina* developed recurrent lesions. Supplementation with

Spirulina did not increase serum concentrations of retinal or beta-carotene, nor was it associated with toxicity. This is the first human study evaluating the chemopreventive potential of *Spirulina*. More studies in different settings and different populations are needed for further evaluations (Mathew, 1995).

Spirulina reduced urine radioactivity levels by 50% in only 20 days. This result was achieved after giving 5 grams a day to children at the Institute of Radiation Medicine in Minsk, Belarus. The Institute has developed a program to treat 100 children every 20 days. This 1993 report confirms 1990-91 research on the beneficial health effects of *Spirulina* on children with radiation sickness. It concludes: "Use of *Spirulina* decreases radioactive dose load received from food contaminated with radionuclides, Cesium-137 and Strontium-90. *Spirulina* is favorable for normalizing the adaptative potential of children's bodies in conditions of long-lived low dose radiation." (Loseva, 1993).

A critical review of data on the safety and therapeutic effects of *Spirulina* was published, ranging from reduction of cholesterol and cancer to enhancing the immune system, increasing intestinal lactobacilli, reducing nephrotoxicity by heavy metals and drugs, and radiation protection (Belay, 1993). *Spirulina* has now been marketed and consumed as a human food in over 60 countries, and approved as a food for human and/or animal consumption by most governments, health agencies and associations (Henrickson, 1989).

(iii) Heavy Metals

Cyanotech Corporation and Earthrise Nutritionals, Inc. have published strict standards for heavy metals in *Spirulina*. A five-year testing program in California showed heavy metals were either not detectable or extremely low. Mercury was not detectable in 40 tests, and the standard for mercury was set at less than 0.05 parts per million (ppm). In comparison, the US FDA standard in 'aquatic animals' is 1.0 ppm, permitting over 20 times more mercury. Standards were set for cadmium (less than 0.05 ppm), lead (less than 1.0 ppm), and arsenic (less than 1.0 ppm) and mercury (less than 0.025 ppm). By comparison, the UN Protein Advisory Group standard for single cell protein permits higher heavy metals: 1.0 ppm for mercury; 1.0 ppm for cadmium,

5.0 ppm for lead; and 2.0 ppm for arsenic. Based on 120 independent lab tests, *Spirulina* producers have set some of the toughest standards for heavy metals (Earthrise Farms. Five year testing of heavy metals in *Spirulina*, 1983-1988).

(iv) Algal Toxin Research Studies

An important quality control issue surrounding production of blue-green algae (Cyanobacteria) is the possibility of inadvertently harvesting other blue-green algae containing cyanotoxins. This is a risk when harvesting algae from natural bodies of water with mixed cultures of microscopic algae. Algal toxins are capable of causing widespread poisoning of animals and humans (Carmichael, 1994).

In 1995-96, a group of leading microalgae producers including Cyanotech Corporation and Earthrise Nutritionals sponsored research conducted by algal toxicologists. The result was a Technical Booklet for the Microalgae Biomass Industry as a guide to the use of a very sensitive enzyme linked immunosorbant assay (ELISA) and a protein phosphate inhibition assay (PPIA) for the detection of toxic microcystins and nodularins. These methods can detect, monitor and control cyanotoxins, so producers can assure a safe, nutritious product for human and animal food supplements (An and Carmichael, 1996).

Spirulina is regularly assayed for microcystin and nodularin toxins by ELISA analysis using in-house testing as well as independent testing at Wright State University, Department of Biological Sciences (3640 Colonel Glen Highway, Dayton, OH, 45425, Ph: 937-775-3173) or at Earthrise Nutritionals Laboratory. Cyanotech Corporation and Earthrise Nutritionals, Inc. have never had any detectable amount of microcystin or nodularin toxins in their *Spirulina* products.

(v) Toxicity, Carcinogenicity, Mutagenicity, Chronic Effects, Dermal Effects

There are no reports of toxicity, carcinogenicity, mutagenicity, chronic or dermal effects from consumption of *Spirulina* documented in the scientific literature. No solvents, pesticides, herbicides or toxic substances are used during any cultivation or manufacturing step of the product. There are no known carcinogens or compounds that may be degraded or metabolized

to carcinogens used in the manufacturing process or known within *Spirulina* powder.

One study was conducted to determine the liver distribution and fecal excretion of polychlorinated dibenzo-p-dioxins (PCDD) congeners in male rats fed with 20% *Spirulina* and other alga. The fecal extraction of the PCDD's was significantly higher in the *Spirulina* group compared with the control group. The experiments suggest that the administration of *Spirulina* or other algae is useful as an approach in the treatment of patients exposed to lipophilic xenobiotics (Morita, 1997).

Spirulina administered to rats orally up to a dosage of 800 mg/kg body weight did not exert any toxic action. No alterations were found in either body or organ weight in treated animals and vital organs showed normal histology. Application of the algae onto skin of albino rats, up to 2000 mg/kg of body weight, did not elicit any skin allergy (Krishnakumari, 1981). Tests to determine if repeated feeding of *Spirulina* (short and prolonged-term) produce germinal lethal mutations in male mice were found to be negative (Chamorro, 1989).

Nutrients are supplied by reliable manufacturers that include specifications for heavy metals and other possible contaminants. No solvents, pesticides, herbicides or toxic substances are used during any cultivation or manufacturing step of the product. Woodson-Tenent Laboratories, Inc. (FDA Registration 1020132, Memphis, TN) and Covance Laboratories are contracted to conduct regular testing of finished products, including proximate analyses, pesticides, heavy metals, and other toxic contaminants. Levels of contaminants have never exceeded FDA specifications.

(vi) Other Approvals of *Spirulina* as Safety Evidence

According to the US Food and Drug Administration, *Spirulina* can be legally marketed in the US as a food as long as it is labeled accurately and contains no contaminated or adulterated substances (Talk Paper, 6/23/82).

(vii) Information that Appears to be Inconsistent for GRAS Determination

The Special Nutritionals Adverse Event Monitoring System (http://vm.cfsan.fda.gov/~dms/aemsfull.html) lists approximately 50 incidents in which *Spirulina* was involved in an adverse event. However, in most of these cases, *Spirulina* was

a minor component and other ingredients such as ma-huang, guarana, or gotu kola could be attributed to causing the symptoms.

A few of the cases are also likely misidentified as *Spirulina* and were actually a wild fresh-water alga harvested from Klamath Lake called, *Aphanizomenon flos-aquae*. This algae is usually marketed as "Super Blue-Green Algae" and is often confused with *Spirulina*. *Aphanizomenon flos-aqua* has been associated with microcystin toxins in recent years.

In one study, male rats fed *Spirulina* from 0-26% of the diet indicated a reduction in plasma, liver and heart tocopherol levels. Weight gain decreased with higher levels of *Spirulina* in the diet (Mitchell, 1990).

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End Submission

CYANOTECH CORPORATION

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28 May 2002

Lane A. Highbarger, Ph.D.
Division of Biotech and GRAS Notice Review, HFS-255
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C Street SW
Washington, DC 20204

Dear Dr. Highbarger,

This is in regard to our GRAS Notice (GRN No. 000101) for *Spirulina* microalgae, submitted 8 March 2002 on behalf of Cyanotech Corporation and Earthrise Nutritionals, Inc. In this notice, we expressed our view, based on scientific procedures, that *Spirulina* is GRAS for use in foods such as bars, nutritional drink mixes, and as a condiment in salads and pasta, at levels ranging from 0.5 to 3 grams per serving. Since submission, we have discussed with yourself and others at CFSAN certain aspects of our notice that may benefit from additional information and clarification.

We therefore respectfully request that you cease to evaluate the present notice, so that we may have an opportunity to provide such clarification and additional information in a new notice. We intend to prepare and submit a new complete GRAS notice for *Spirulina*, and to incorporate by reference to GRN No. 000101 those supporting documents contained therein.

Thank you very much for your attention to our present and future notices.

Sincerely,

John E. Dore, Ph.D.
Scientific Director, Cyanotech Corporation

Authorized Agent for Notifiers:

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