ORIGINAL SUBMISSION

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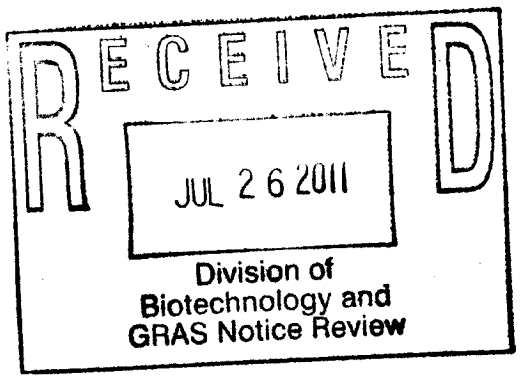

July 21, 2011

Dr. Susan Carlson
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

Re: GRAS exemption claim for *Spirulina platensis* as an ingredient in foods

Dear Dr. Carlson,

This is to notify you that RFI, Inc. claims that the use of the substance described below (*Spirulina platensis*) is exempt from the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act because RFI has determined such use to be Generally Recognized As Safe (GRAS).

On behalf of RFI, NutraSource (an independent consulting firm) assembled a panel of experts highly qualified by scientific training and experience to evaluate the safety of the intended uses of *Spirulina platensis*. The panel included Dr. Susan Cho at NutraSource (Clarksville, MD), Dr. Joanne Slavin at the University of Minnesota (St. Paul, MN), and Dr. George Fahey at the University of Illinois (Urbana, IL). Following independent critical evaluation of the available data and information, the panel has determined that the use of *Spirulina platensis* (that is manufactured by RFI) described in the enclosed notification is GRAS based on scientific procedures.

After reviewing the available data, the Expert Panel concluded in its July 2011 statement that the intended use of RFI's *Spirulina platensis* (to be used as an ingredient in foods such as granola bars, cereal bars, protein bars and power bars, meal replacements and mixes, sports beverages, energy drinks, energy soft drinks, fruit juices, low calorie fruit and vegetable juice drinks, low fat soy milk, and medical foods), resulting in an estimated 90th percentile daily intake of 1.35 g, is safe and GRAS for the users of *Spirulina platensis*.

This determination and notification are in compliance with proposed Sec. 170.36 of Part 21 of the Code of Federal Regulations (21 CFR section170.36) as published in the Federal Register, Vol. 62, No. 74, FR 18937, April 17, 1997.

Notifier's name and Address: RFI, Inc.
300 Corporate Drive, suite 14, Blauvelt, NY 10913
Attention: Mr. Paul Altaffer
Phone number: 415-334-7199
Fax number: 415-334-7395
E mail address: Paulo@rfiingredients.com

Name of GRAS substance: *Spirulina platensis* (conventional and organic), *Spirulina,* organic *Spirulina*, or *Arthrospira platensis.*

Product description:
Spirulina (Arthrospira), a class of cyanobacteria, is a free-floating filamentous microalgae which is capable of photosynthesis. The nutritional value of *Spirulina* is well recognized with its exceptionally high content of protein (60–70% by dry weight), vitamins, minerals, essential fatty acids, and other nutrients. The 2008 FAO position paper describes *Spirulina* as follows: "An easily digestible high (c. 60%) protein product with high levels of beta-carotene, vitamin B12, iron and trace minerals, and the rare essential fatty acid y-linolenic acid [also called gamma-linolenic acid (GLA), or omega-6". Over the history of safe use of Spirulina, it has been generally recognized as safe (GRAS) for human consumption. Human clinical studies and animal studies over the past several decades support such notion. Also, FDA had no question on the GRAS notice (GRN 127) of *Spirulina platensis* (FDA, 2003).

Applicable conditions of use of the notified substance:
The proposed use levels of *Spirulina platensis* are presented in Table 1.

Table 1. Proposed food application of *Spirulina platensis* and maximum levels of use

Proposed food use	Serving size, g	Use level, g/serving	Use level, %
Granola bars, cereal bars, protein bars, and power bars	30	1.5	5
Meal replacement and mixes	240	3.6	1.5
Sports beverages	240	3.6	1.5
Energy drink	40	3.6	9
Energy soft drinks	240	3.6	1.5
Fruit juices, such as lime juice, blackberry juice, grape juice; low calorie fruit and vegetable juice drinks	240	3.6	1.5
Low fat soy milk	240	3.6	1.5
Medical foods	120	12	10

Intended use includes granola bars, cereal bars, protein bars and power bars, meal replacements and mixes, sports beverages, energy drinks, energy soft drinks, fruit juices, low calorie fruit and vegetable juice drinks, low fat soy milk, and medical foods. As described in GRN 000127, *Spirulina platensis* is not intended for use in meat or poultry-containing products or as a coloring agent.

Assuming that 100% of the product will be used at the maximum levels under the intended use, the 90th percentile intakes from the intended use by users of one or more foods are 13.5 g/d (208 mg/kg BW/d) for the population aged 1 year and above (combining males and females), 15.5 g/d (241 mg/kg BW/d) for males, and 7.5 g/d (139 mg/kg BW/d) for females. After adjustments for market shares (10% of the market share), the 90th percentile intakes by users of one or more foods are 1.35 g/d (20.8 mg/kg BW/d) for the population combining males and females, 1.55 g/d (or 24.1 mg/kg BW/d) for males, and 0.75 g/d (13.9 mg/kg BW/d) for females.

Even if all of the products are used at the maximum levels, exposure estimate levels are much lower than the no-observed-adverse-effect level (NOAEL) values (10,000 mg/kg BW/d) that have been found from toxicity studies in animals and a proven safe intake level of 4,132 mg/kg BW/d which has been found from human clinical trials.

Basis of GRAS determination: Through scientific procedures.

Review and copying statement:
The data and information that serve as the basis for this GRAS determination will be sent to the FDA upon request, or are available for the FDA's review and copying at reasonable times at the office of RFI, Inc. or Nutrasource, Inc.

We enclose an original and two copies of this notification for your review. If you have any questions, please contact me.

Sincerely,

(b) (6)

Susan Cho, Ph.D.
Chief Science Officer

Executive Summary

The objective of this Generally Recognized as Safe (GRAS) determination is to summarize the available safety information on *Spirulina platensis*, which is used as an ingredient in foods and beverages.

We, the undersigned expert panel members, Susan Cho, Ph.D., Joanne Slavin, Ph.D., and George C. Fahey, Jr., Ph.D., have individually and collectively critically evaluated the materials summarized in the *Spirulina platensis* GRAS report. We conclude that *Spirulina platensis* is safe and GRAS for its intended use in food. There is broad-based and widely disseminated knowledge concerning the chemistry and health benefits of *Spirulina platensis* in both human and animals.

Pursuant to 21 CFR § 170.30, this GRAS determination for *Spirulina platensis* is based on scientific procedures. There are no indications of significant adverse effects related to *Spirulina platensis* in the publicly available literature, and the manufacturing process of *Spirulina platensis* does not employ any treatments with organic solvents. In the United States, *Spirulina platensis* has been already recognized as a GRAS substance since 2003 (FDA, GRN 000127). Since that time, several toxicity and human clinical studies have been published to report higher values of safe intake levels than the previously reported. This GRAS notice captures the findings from recent studies.

Documentation qualifying a substance as GRAS has been compiled. Such documentation includes technical evidence and common knowledge of safety, as recognized by qualified experts (the Expert Panel). Technical evidence of safety includes the chemical identity of the substance, the method of manufacture, analytical data on composition and specifications, safety data from animal and human clinical studies, and nutritional benefits from animal and human clinical studies. *Spirulina platensis* belongs to the “practically nontoxic” category, according to a toxicity rating chart.

Intended use includes granola bars, cereal bars, protein bars and power bars, meal replacements and mixes, sports beverages, energy drinks, energy soft drinks, fruit juices, low calorie fruit and vegetable juice drinks, low fat soy milk, and medical foods. As described in GRN 000127, *Spirulina platensis* is not intended for use in meat or poultry-containing products or as a coloring agent.

Assuming that 100% of the product will be used at the maximum levels under the intended use, the 90th percentile intakes from the intended use by users of one or more foods are 13.5 g/d (208 mg/kg BW/d) for the population aged 1 year and above (combining males and females), 15.5 g/d (241 mg/kg BW/d) for males, and 7.5 g/d (139 mg/kg BW/d) for females (Table 3). After adjustments for market shares, the 90th percentile intakes by users of one or more foods are 1.35 g/d (20.8 mg/kg BW/d) for the population combining males and females, 1.55 g/d (or 24.1 mg/kg BW/d) for males, and 0.75 g/d (13.9 mg/kg BW/d) for females.

Even if all of the products are used at the maximum levels, exposure estimate levels are much lower than the no-observed-adverse-effect level (NOAEL) values (10,000 mg/kg BW/d) that have been found from toxicity studies in animals and a proven safe intake level of 4,132 mg/kg BW/d which has been found from human clinical trials.

Therefore, the proposed use of *Spirulina platensis* is not only safe within the terms of the Federal Food, Drug, and Cosmetic Act (meeting the standard of reasonable certainty of no harm), but it is also GRAS according to Title 21 Code of Federal Regulations (21 CFR) because of this consensus among experts.

Susan Cho, Ph.D.
President, NutraSource, Inc., Clarksville, MD 21029

Signature: _____ Date: _____

Joanne Slavin, Ph.D., R.D.
Professor, University of Minnesota, St. Paul, MN 55108
Signature: _____ Date: _____

George C. Fahey, Jr., Ph.D.
Professor Emeritus, University of Illinois, Urbana, IL 61801

Signature: _____ Date: _____

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President, NutraSource, Inc., Clarksville, MD 21029
Signature: (b) (6) Date: 7/20/2011

Joanne Slavin, Ph.D., R.D.
Professor, University of Minnesota, St. Paul, MN 55108
Signature: _____ Date: _____

George C. Fahey, Jr., Ph.D.
Professor Emeritus, University of Illinois, Urbana, IL 61801
Signature: (b) (6) Date: 7/18/11

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Signature: _____ Date: _____

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Professor, University of Minnesota, St. Paul, MN 55108

Signat (b) (6) _____ Date: 2-11-11

George C. Fahey, Jr., Ph.D.
Professor Emeritus, University of Illinois, Urbana, IL 61801

Signature: _____ Date: _____

I. Identity of Substance

A. Common or trade name: Spirulina, organic Spirulina, *Spirulina platensis,* Arthrospira*,* or *Arthrospira platensis.*

B. Standards of identity: We note that an ingredient that is lawfully added to food products may be used in a standardized food only if it is permitted by the applicable standard of identity that is located in Title 21 of the Code of Federal Regulations.

C. Background

Spirulina (Arthrospira), a class of cyanobacteria, is a free-floating filamentous microalgae which is capable of photosynthesis (Ciferri and Tiboni, 1985; Komarek et al., 2009; Sapp, 2005). Spirulina naturally grows in high-salt alkaline water reservoirs in subtropical and tropical areas including Hawaii, Mexico, Asia, and Central Africa (Gershwin and Belay, 2008). Among the Spirulina species, *Spirulina platensis* (*Arthrospira platensis*), *Spirulina maxima* (*Arthrospira maxima*), and *Spirulina fusiformis* (*Arthrospira fusiformis*) have been most intensively studied (Gershwin and Belay, 2008; Karkos et al., 2008; Khan et al., 2005).

The nutritional value of Spirulina is well recognized with its exceptionally high content of protein (60–70% by dry weight), vitamins, minerals, essential fatty acids, and other nutrients (Annapurna et al., 1991; Deng et al., 2010; McCarty, 2010). The 2008 FAO position paper describes Spirulina as follows: "An easily digestible high (c. 60%) protein product with high levels of beta-carotene, vitamin B12, iron and trace minerals, and the rare essential fatty acid y-linolenic acid [also called gamma-linolenic acid (GLA), or omega-6".

Over the history of safe use of Spirulina*,* it has been generally recognized as safe (GRAS) for human consumption. Human clinical studies and animal studies over the past several decades support such notion. Thus, FDA had no question on the GRAS notice (GRN 127) of *Spirulina platensis* (FDA, 2003).

D. General properties of *Spirulina platensis*

Spirulina is a free-flowing, dark blue-green powder with a mild seaweed smell, produced by spray drying the biomass of the cyanobacterium, *Arthrospira platensis* (Dillon et al. 1995). It is not readily soluble in water or solvents, but it forms a suspension when mixed with water.

E. Manufacturing Process

E.1. Conventional Spirulina

1. Selection and culture preparation of Spirulina seed in the lab: The certified culture obtained from the Academy of Sciences of China is cultured in the lab in media using ultra-high purity fertilizer in triangular flasks and glass bottles. These

are cultivated at 18-38°C.
2. Cultivation in Spirulina farming pool: The cultured Spirulina seed is cultivated in large ponds, which contains food grade fertilizers (like sodium bicarbonate, potassium chloride, potassium dihydrogen phosphate, and salt), water, and carbon dioxide. The Spirulina is harvested after a series of QC tests are performed including microscopic testing.
3. Spray drying: The harvested Spirulina then is dried using a spray drying system after washing with water to bring the pH to neutral from alkaline. The Spirulina droplets are sprayed into the chamber from the top of the tower to flash evaporate the water.
4. Packaging: The Spirulina powder then is packaged.

E.2. Organic Spirulina

1. For organic Spirulina, Spirulina seeds are cultivated at 18-38°C with ultra-high purity fertilizer in triangular flasks and glass bottles.
2. The cultured Spirulina seed is cultivated in large ponds, which contains organic fertilizers (non-allergenic soy dregs, non-allergenic peanut dreg, humic acid, natural potassium ore powder, and CO2), water, and carbon dioxide.
3. The Spirulina is harvested after a series of QC tests are performed including microscopic testing.
4. The harvested Spirulina then is dried using a spray drying system after washing with water to bring the pH to neutral from alkaline. The Spirulina droplets are sprayed into the chamber from the top of the tower to flash evaporate the water.
5. The Spirulina powder then is packaged.

F. Specifications

Tables 1a and 1b lists specifications of conventional and organic Spirulina.

Table 1a. Specifications of conventional Spirulina powder

Product Name: Spirulina Powder (conventional)
Botanical Name: *Arthrospira platensis*
Product Code: RFI-GC130018
Carrier/Preservatives: None
Country of Origin: China

Characteristic	Test Method	Specification
Appearance	Visual	Fine green powder
Identification	FTIR/scientific method	Complies to standard
Sensory	Organoleptic	Mild like sea weed
Particle size	AOAC 973.03 (TQ-106)	100% through 80 mesh
Moisture	TQ-104 (104°C/2h)	≤ 8.0%
Protein	AOAC 968.06/945.18	≥ 60%
Carotenoids	AOAC 955.10/ Paper chromatography	≥ 4 mg/g
Ash	AOAC 923.03	≤ 8.0%
Crude phycocyanin		12-18%
Heavy metal		
Lead	ICP/MS AOAC 993.14	≤ 1.0 ppm
Arsenic	ICP/MS AOAC 993.14	≤ 0.5 ppm
Cadmium	ICP/MS AOAC 993.14	Informative, test annually or every 5 lots
Mercury	ICP/MS AOAC 993.14	Informative, test annually or every 5 lots
Chromium VI	SW846/7196A (EPA)	Informative, test annually or every 5 lots
Microbiology		
Aerobic plate count	AOAC 966.23	≤ 100,000 cfu/g
E. coli	USP33, NF28, 2010	Negative/10 g
Salmonella	USP33, NF28, 2010	Negative/10 g
Staphylococcus aureus	USP33, NF28, 2010	Negative/10 g
Coliforms	AOAC 966.24	≤ 10 cfu/g
Yeast/mold	FDA-BAM, 7th Ed.	≤ 300 cfu/g
Pesticides	FDA 302	Informative, test annually or every 5 lots
Aflatoxins	AOAC 991.31 (HPLC)/ AOAC 990.34 (AFLA-20 Cup Test Kit)	≤ 20 ppb, test annually or every 5 lots

*This is a natural product and there could be color and taste variations from lot to lot due to crop fluctuations from harvest to another.

Table 1b. Specifications of organic Spirulina powder

Product Name: Spirulina Powder - Organic
Botanical Name: *Arthrospira platensis*
Product Code: RFI-GO130001
Plant Part: Whole plant
Carrier: None
Country of Origin: China

Characteristic	Test Method	Specification
Appearance	Visual	Fine dark green powder
Identification	FTIR/scientific method	Complies to standard
Protein	AOAC 968.06/945.18	≥ 60%
Chlorophyll	UV Spectrophotometry	11-14 mg/g, test semi-annually
Carotenoids	AOAC 924.04/Paper chromatography	4.0-5.5 mg/g, test semi-annually
Moisture	TQ-104 (104°C/2h)	≤ 8.0%
Particle size	AOAC 973.03 (TQ-106)	100% pass on 80 Mesh
Ash	AOAC 930.03	≤ 8.0%
Crude phycocyanin	UV Spectrophotometry	12-19%, test semi-annually
Heavy Metals		
Lead	ICP/MS AOAC 993.14	≤ 1.0 ppm
Arsenic	ICP/MS AOAC 993.14	≤ 0.5 ppm
Cadmium	ICP/MS AOAC 993.14	Informative, test annually (ppm)
Mercury	ICP/MS AOAC 993.14	Informative, test annually (ppm)
Chromium VI	SW846/7196A (EPA)	Informative, test annually (ppm)
Microbiology		
Aerobic plate count	AOAC 966.23	≤ 100,000 cfu/g
E. coli	USP33, NF28, 2010	Negative/10g
Salmonella	USP33, NF28, 2010	Negative/10g
Staphylococcus aureus	USP33, NF28, 2010	Negative/10g
Coliforms	AOAC 966.24	≤ 10 cfu/g
Yeast and mold	*FDA-BAM 7th ed.*	≤ 300 cfu/g
Aflatoxins	AOAC 991.31 (HPLC)/ AOAC 990.34 (AFLA-20 Cup Test Kit)	≤ 20 ppb, test annually

*This is a natural product and there may be color and taste variations from lot to lot due to crop fluctuations from harvest to harvest.

Quality control process

After harvest, the products, product was washed several times, and then spray dried at high temperature. End product control: every lot of product is tested for microbial, heavy

metal, and foreign material contamination. The product must pass evaluation before it is released.

An important quality control issue surrounding production of cyanobacteria is the possibility of inadvertently harvesting other cyanobacteria containing cyanotoxins. This is a risk when harvesting algae from natural bodies of water containing mixed populations of phytoplankton, but is unlikely to be a problem with the tightly controlled *Arthrospira platensis* monocultures utilized by the notifier. However, Spirulina is periodically assayed for microcystin and nodularin toxins by ELISA analysis using in-house testing as well as independent testing.

II. Natural occurrence and exposure to *Spirulina platensis*

A. Use of Spirulina as a foodstuff

Spirulina has been safely consumed as a food ingredient in Mexico and Central Africa for the past four centuries and is currently used as a dietary supplement and a novel food ingredient, especially among Asians, including Asian-Americans (Ciferri and Tiboni, 1985).

B. Intended use

Table 2 presents primary applications of *Spirulina platensis* that include granola bars, cereal bars, protein bars and power bars, meal replacements and mixes, sports beverages, energy drinks, energy soft drinks, lime juice, blackberry juice, grape juice, low calorie fruit and vegetable juice drinks, low fat soy milk, and medical foods. As described in GRN 000127, *Spirulina platensis* is not intended for use in meat or poultry-containing products or as a coloring agent.

Table 2. Intended use of *Spirulina platensis*

Proposed food use	Serving size, g	Use level, g/serving	Use level, %
Granola bars, cereal bars, protein bars, and power bars	30	1.5	5
Meal replacement and mixes	240	3.6	1.5
Sports beverages	240	3.6	1.5
Energy drink	40	3.6	9
Energy soft drinks	240	3.6	1.5
Fruit juices, such as lime juice, blackberry juice, grape juice; low calorie fruit and vegetable juice drinks	240	3.6	1.5
Low fat soy milk	240	3.6	1.5
Medical foods	120	12	10

C. Probable consumption of *Spirulina platensis*

Using food intake data reported in the 2005-2008 NHANES, exposure levels to *Spirulina platensis* that will result from the intended uses were estimated. Results of the exposure estimates under the intended use of *Spirulina platensis* in foods for the U.S. population ages 1 y and older are presented in Tables 3 and 4.

The first set of estimates is based on the assumption that all the products in each food category will be used at the maximum intake levels under the intended use although it is far from a realistic situation. The 90th percentile intakes from the intended use by users of one or more foods are 13.5 g/d (208 mg/kg BW/d) for the population aged 1 y and above (combining males and females), 15.5 g/d (241 mg/kg BW/d) for males and 7.5 g/d (139 mg/kg BW/d) for females (Table 3).

The second set of estimates is based on the market adjustment which assumes that 10% of the products in each food category will be used at the maximum intake levels under the intended use (Table 4). From a marketing perspective, an assumption that 10% of the product will be used at the maximum levels for each food category is a highly optimistic projection. It is not possible to use all the foods under the intended use. Also, wastage and other losses should be considered. The 90th percentile intakes by users of one or more foods are 1.35 g/d (20.8 mg/kg BW/d) for the population combining males and females, 1.55 g/d (or 24.1 mg/kg BW/d) for males, and 0.75 g/d (13.9 mg/kg BW/d) for females.

These estimated daily exposure levels are far lower than the no-observed-adverse-effect level (NOAEL) values (*S. platensis*, 10,000 mg/kg BW/d, the maximum levels tested; other *Spirulina* species, 30,000 mg/kg BW/d) that have been found from various toxicity studies in rats. Also, these levels are far below the safe use level of 4,132 mg/kg BW/d that has been found from human clinical studies (Simpore et al., 2005, 2006).

Medical foods have not been included in the exposure estimate of the general population since those foods will be used by a small segment of special population.

Table 3a. Exposure estimate for *Spirulina platensis* intakes assuming 100% of products will be used at the maximal levels under the intended use, g/d.

Age, y	All population			Users				
	N	Mean	SE	% of population	Mean	SE	90th	SE
1+ all	17635	0.67	0.042	11.85	5.62	0.21	13.45	0.89
1+ males	8720	1.00	0.075	13.56	7.41	0.35	15.46	0.87
1+ females	8915	0.35	0.020	10.24	3.41	0.11	7.49	0.43
1-3	1521	0.29	0.039	10.24	2.85	0.29	5.61	1.12
4-12	3324	0.58	0.061	14.08	4.15	0.32	9.15	0.46
13-19, all	2851	1.30	0.135	16.79	7.74	0.58	14.64	0.90
13-19, males	1432	1.88	0.249	18.11	10.38	0.73	18.32	3.10
13-19, females	1419	0.71	0.091	15.46	4.60	0.29	10.97	0.68
20+ all	9939	0.61	0.045	10.88	5.64	0.28	13.74	0.90
20+ males	4824	0.97	0.087	12.88	7.55	0.47	16.30	1.36

Table 3b. Exposure estimate for *Spirulina platensis* intakes assuming 100% of products will be used at the maximal levels under the intended use, mg/kg BW/d.

Age, y	All population			Users				
	N	Mean	SE	% of population	Mean	SE	90th	SE
1+ all	17635	10.90	0.62	11.85	91.96	3.17	207.62	9.10
1+ males	8720	15.20	1.00	13.56	111.90	4.64	240.89	15.00
1+ females	8915	6.86	0.42	10.24	67.039	3.15	139.22	10.42
1-3	1521	22.52	3.27	10.24	221.44	24.74	448.68	110.5
4-12	3324	18.46	1.76	14.08	131.70	8.80	287.60	16.89
13-19, all	2851	20.28	2.30	16.79	121.15	9.87	226.16	23.44
13-19, males	1432	28.31	4.18	18.11	155.97	14.04	277.52	79.79
13-19, females	1419	12.01	1.47	15.46	78.52	4.49	164.86	17.87
20+ all	9939	7.67	0.54	10.88	70.32	3.10	158.45	13.94
20+ males	4824	11.77	1.00	12.88	91.07	5.47	201.85	19.42

Table 4a. Exposure estimate for *Spirulina platensis* intakes after the market share adjustment (assuming that 10% of the products in each food category will be used at the maximum intake levels under the intended use), g/d

Age, y	All population			Users				
	N	Mean	SE	% of population	Mean	SE	90th	SE
1+ all	17635	0.066	0.004	11.85	0.56	0.021	1.35	0.089
1+ males	8720	0.10	0.007	13.56	0.74	0.035	1.55	0.087
1+ females	8915	0.035	0.002	10.24	0.34	0.011	0.75	0.043
1-3	1521	0.029	0.004	10.24	0.28	0.029	0.56	0.111
4-12	3324	0.058	0.006	14.08	0.42	0.032	0.91	0.046
13-19, all	2851	0.130	0.013	16.79	0.77	0.058	1.46	0.090
13-19, males	1432	0.19	0.025	18.11	1.04	0.073	1.83	0.310
13-19, females	1419	0.071	0.009	15.46	0.46	0.029	1.10	0.068
20+ all	9939	0.061	0.004	10.88	0.56	0.028	1.37	0.090
20+ males	4824	0.097	0.009	12.88	0.76	0.048	1.63	0.136

Table 4b. Exposure estimate for *Spirulina platensis* intakes after the market share adjustment, mg/kg BW/d

Age, y	All population			Users				
	N	Mean	SE	% of population	Mean	SE	90th	SE
1+ all	17635	1.09	0.062	11.85	9.20	0.32	20.8	0.91
1+ males	8720	1.52	0.100	13.56	11.19	0.46	24.1	1.50
1+ females	8915	0.69	0.042	10.24	6.70	0.31	13.9	1.04
1-3	1521	2.25	0.327	10.24	22.14	2.47	44.9	11.05
4-12	3324	1.85	0.176	14.08	13.17	0.88	28.8	1.69
13-19, all	2851	2.03	0.230	16.79	12.11	0.99	22.6	2.34
13-19, males	1432	2.83	0.418	18.11	15.60	1.40	27.8	7.98
13-19, females	1419	1.20	0.147	15.46	7.85	0.45	16.5	1.79
20+ all	9939	0.77	0.054	10.88	7.03	0.31	15.8	1.39
20+ males	4824	1.18	0.010	12.88	9.11	0.55	20.2	1.94

III. Basis for GRAS determination

A. Current regulatory status.

Spirulina platensis has been classified as a GRAS substance by the United States Food and Drug Administration (GRN 000127; FDA, 2003).

B. Intended technical effects

Spirulina platensis can be used as an ingredient in foods and beverages as a food ingredient. *Spirulina platensis* is not intended for use as a food colorant.

C. Review of safety data

1. Animal Studies of Spirulina

Safety evaluations on Spirulina sp. have included acute, subchronic, chronic, teratogenic, mutagenic, carcinogenic, and multiple generation effects. No signs of toxicity were noted in any of these studies. The NOAELs appear to be over 10,000 mg/kg BW/d for *Spirulina platensis* and 30% in the diet for Spirulina sp (corresponding to 30,000 mg/kg BW/d), the maximum levels tested (Tables 5 - 7). Overall, Spirulina has high NOAEL values.

1.1. **Acute Toxicity Tests** (Table 5)

Dried Spirulina at the dosage of 10,000 mg/kg BW showed no toxic effects. Some authors recommend accompanying this test with a histological analysis similar to the one conducted with phycocyanin, an active component of Spirulina. The LD_{50} value for phycocyanin was found to be >5,000 mg/kg (Naidu et al., 1999). Since the 1990s, these high LD_{50} values (over 5,000 mg/kg BW) belong to the “practically nontoxic” category, according to a toxicity rating chart (Atlug, 2003).

1.1.1. Acute toxicity of *S. platensis* in mice

Hutadilok-Towatana et al. (2008) tested acute effects of *Spirulina platensis*. Mice that were fed with *Spirulina platensis* at the dose of 30 g/kg BW fresh algae or 10 g/kg BW dried algae did not have any signs of toxicity during 7 d of observation. There were no obvious differences between treatment and the control groups. The gross examinations of internal organs revealed no pathological abnormalities. These results suggest that *Spirulina platensis* is not toxic to mice after exposure to a high dose (dried algae, 10,000 mg kg BW/d) for 7 d (Hutadilok-Towatana et al., 2008).

Krishnakumari et al. (1981) reported that oral treatment with 800 mg/kg *Spirulina platensis* produced no toxic effects on BW, organs, and histological parameters. Also, application of 2,000 mg/kg onto the skin of albino rats did not elicit an allergic skin reaction (Krishnakumari et al., 1981).

Table 5. Acute toxicity studies of *Spirulina platensis*

Species	Dose	Duration	NOAEL	
Mice	30,000 mg/kg fresh algae or 10,000 mg/kg dried algae	7 d	No toxicity	Hutadilok-Towatana, 2008
Rat	800 mg/kg BW dried algae	Single dose	No toxicity	Krishnakumari et al., 1981
Rat	2,000 mg/kg BW dried algae	Single application	No skin allergic reaction	Krishnakumari et al., 1981

1.2. **Subchronic Toxicity Studies** (Table 6)

The consumption of dried Spirulina at doses up to 300 mg/kg/d for 12 wk did not show any adverse effects in rats (Hutadilok-Towatana et al., 2008; Krishnakumari et al., 1981)

Table 6. Subchronic toxicity studies of *Spirulina platensis*

Species	Dose	Duration	NOAEL	
Rat	Dried algae, up to 120 mg/kg BW/d	12 wk	120 mg/kg BW/d	Hutadilok-Towatana et al., 2008
Rat	Fresh algae, up to 1,200 mg/kg BW/d	12 wk	1,200 mg/kg BW/d	Hutadilok-Towatana et al., 2008
Rat	Dried algae, 300 mg/kg BW/d	12 wk	300 mg/kg BW/d	Krishnakumari et al., 1981

1.2.1. Subchronic toxicity studies of *Spirulina platensis* by Hutadilok-Towatana et al. (2008)

Two separate experiments were performed to evaluate subchronic toxicity of fresh or dried forms of *Spirulina platensis.* In each experiment, four groups of six Sprague-Dawley male and female rats were given fresh (up to 1,200 mg/kg BW/d) or dried algae (up to 120 mg/kg BW/d) for 12 wk. The consumption of algae showed no effect on behavior, food and water intake, growth or health status of these animals during the course of this investigation. There were no significant differences in serum clinical chemistry values and all values remained within the normal ranges (Casey and King, 1980; Angkhasirisap et al., 2002) and no treatment effects were noted.

There were no significant differences in BW gains, final BW, or fecal characteristics of rats among all treatment and control groups. Such findings indicate that *Spirulina platensis* did not alter protein, carbohydrate, or fat utilization, or gastrointestinal effects in rats.

To determine if rats treated with fresh *Spirulina platensis* had any intravascular effects and bone marrow activity, Hutadilok-Towatana et al. (2008) performed hematological examinations. The average hematocrit values in all female groups appear to be lower than those in the males. However, feeding the rats with fresh algae significantly improved hematological parameters.

At autopsy, macroscopic observation of the organs did not show any abnormality in their gross appearances or weights due to the consumption of *Spirulina platensis*. There were no changes in morphology and no unusual lesions in the gastrointestinal tissues exposed to *Spirulina platensis*.

In addition, post-mortem examination found no abnormalities in the gross findings. The results demonstrated that short-term and long-term consumption of *Spirulina platensis*, up to high feeding levels, did not produce any adverse effects in experimental animals.

1.2.2. Subchronic toxicity of *Spirulina platensis* by Becker et al (1980).

Becker et al. (1980) demonstrated that feeding *Spirulina platensis* at 300 mg/kg BW/d did not cause any undesirable effects to the experimental animals.

1.2.3. Subchronic toxicity of other types of Spirulina

As shown in Table 7, subchronic toxicity studies of other types of Spirulina (either unidentified species or *S.maxima*) did not show any treatment-associated effects on their BW gains or final weights in experimental animals. There were no significant differences among the treated and control groups of the same sex. Such findings thus indicated that Spirulina, in general, did not alter protein, carbohydrate, or fat utilization in rats and mice (Salazar et al., 1998). The NOAEL appears to be over 30% Spirulina (or 30,000 mg/kg BW/d) in the diet, the maximum level tested in those studies.

Table 7. Subchronic toxicity studies of other sources of Spirulina (unidentified species or *S. maxima)*

Animals	Concentration in diet	Duration	Results	References
Rats	10% Spirulina (unidentified species); 10, 20, and 30% (corresponding to up to 30,000 mg/kg BW/d)	12 wk	NOAEL, 30% in the diet. Spirulina group had insignificantly lower weight gains compared to a casein control group. All rats survived the experimental period in apparently good health. All organs were normal macroscopically and microscopically.	Becker and Venkataraman, 1984
Rats	Spirulina-rich (unidentified species) diets, at the final conc. of 36 and 48% of protein	100 d	No histological abnormalities were found in several organs examined. Tolerance was good.	Bourges et al., 1971
Rats	Spirulina (unidentified species) at the conc. of 14.25% protein	18 mo	No obvious toxicity signs.	Boudene et al., 1975
Rats	Spirulina (unidentified species); 10, 20, and 30%(corresponding to up to 30,000 mg/kg BW/d)	6 mo	NOAEL, 30% in the diet. Abnormal findings were not detected in growth and external appearance of the whole body or in the shape, weight, and histological findings of organs. Hematological tests such as Hb and SGPT showed some statistical differences	Khan, 2006
Rats	*S.maxima*; 10, 20, and 30% in diet (corresponding to up to 30,000 mg/kg BW/d)	13 wk for F3 gene-ration	NOAEL, 30% in the diet. No toxic effects on food consumption, BW, hematology, clinical chemistry, urine analyses, organ weights, histology or renal function tests	Chamorro et al., 1988[a]
Mice	*S.maxima*; 10, 20, and 30% in diet (corresponding to up to 30,000 mg/kg BW/d)	13 wk	NOAEL, 30% in the diet. No effects on mouse behavior, food and water intake, growth, or survival. Hematology, clinical chemistry, and histopathology values did not reveal differences compared to the control animals.	Salazar et al., 1998

**Modified from Chamorro et al., 2008; Hb=hemoglobin; SGPT= serum glutamic pyruvic transaminase

1.3. Chronic Toxicity Tests

Chamorro et al. (1988[b]) performed chronic toxicity tests of *Spirulina maxima* (10, 20, and 30% in diet) for 86 wk to assess the cumulative toxicity and carcinogenic potential of Spirulina. The survival of animals treated with the Spirulina was equal or slightly higher than that of soy-fed controls. No adverse effects on hematology, urine, macroscopic or histopathological findings, or serum biochemistry were observed. Spirulina at the concentration of 30% in diet did not impact palatability and did not induce diminished food consumption (Chamorro et al., 1988[b]). The NOAEL was determined to be 30% in the diet.

1.4. Reproductive Toxicity Tests (Table 8)

1.4.1. Reproductive Performance of *Spirulina platensis*

Kapoor and Mehta (1993, 1998) studied the effects of *Spirulina platensis* on the outcome of pregnancy. As shown in Table 8, treatment with *Spirulina platensis* at the final concentration of 22% protein was not associated with any adverse effects in any measure of reproductive performance, including food intake, weight gain, numbers of pups, total litter weight, mean birth weight, or hematological status.

Table 8. Reproductive toxicity studies showing no adverse effects of *Spirulina platensis*

Animals	Dose	Duration	Test parameters	Reference
Rats	*S. platensis* at a final conc. of 22% protein		Fertility and pregnancy; food intake, weight gain, numbers of pups, total litter wt, and mean birth wt.	Kapoor and Mehta (1993)
Rat	*S. platensis* at a final conc. of 22% protein	During pregnancy and lactation	Hematological status during pregnancy and lactation	Kapoor and Mehta (1993)
Japanese quail	0, 1.5, 3.0, 6.0, and 12.0% of diet	33 wk	Growth, egg production, egg quality, fertility, hatchability and the growth of the F1 generation of dams	Ross and Dominy (1990)

In the study of Kapoor and Mehta (1993), pregnant rats were fed 5 different kinds of diets providing 22% protein (casein, Spirulina, wheat gluten, Spirulina + wheat gluten, Spirulina without additional vitamins and minerals) during the period of pregnancy. The outcome of pregnancy was assessed from litter and dams' weight and litter size. Maternal weight gain was found to be maximum with Spirulina + wheat gluten and least with the wheat gluten diet. Litter size was higher with Spirulina diets than casein and wheat gluten diets. There no significant differences in food intake and pup's birth weight among the groups. No adverse effects of Spirulina were noted. Authors concluded that Spirulina appeared to be a good dietary supplement during pregnancy.

Kapoor and Mehta (1993b) also studied the effects of *Spirulina platensis* on hematological status in rats during pregnancy and lactation. As shown in Table 8, diets containing Spirulina alone or in combination with wheat gluten resulted in significantly higher iron storage and hemoglobin (Hb) content compared to casein and wheat gluten diets during the first half of pregnancy and lactation. The values for serum iron and iron binding capacity remained unchanged with different diets. Spirulina appears to be effective in improving the iron status of rats during pregnancy and lactation.

1.4.2. Effects of *S. platensis* on reproductive performance of Japanese quail

Ross and Dominy (1990) studied the effects of *S. platensis* (0, 1.5, 3.0, 6.0, and 12.0% of diet for 33 wk) on growth, egg production, egg quality, fertility, hatchability, and the growth of the F1 generation of dams fed Spirulina in Japanese quail. There were no significant differences due to the Spirulina content in any of the parameters studied, except for yolk color (which increased with each succeeding level of Spirulina; 12% *S. platensis*, 2.2 vs. control, 4.9 Roche scale score, $p<0.05$) and for fertility (which was higher for all Spirulina treatments versus the control; 12% *S. platensis*, 95.1 vs. control, 86.8%, $p<0.05$).

These data are consistent with findings from *Spirulina maxima* studies. *Spirulina maxima* was not associated with any adverse effects in any measure of reproductive performance and fetus developmental markers except for isolated results with no toxicological significance (Chamorro et al., 1985, 1988[c], 1989[b], 1997; Romeo-Manilla et al., 2008; Salazar et al., 1996, 1997). Spirulina, even at a 30% dietary concentration, did not result in reproductive toxicity.

Teratogenic studies showed that Spirulina given during different gestation periods did not affect embryo development or produce embryo resorption in rats and mice.

1.4.3. Effects of other types of Spirulina (*S. maxima*) on reproductive performance

In addition, two multi-generational studies showed that Spirulina given to three generations over a period of approximately 2 years showed no adverse effects from Spirulina on fertility or gestation or any other parameters (Table 9; Chamorro et al., 1985, 1988[c]). It is noteworthy that F3 generation rats that were maintained for 13 wk on the same Spirulina diets as their parents' had no adverse effects on food consumption, BW, hematology, clinical chemistry, urine analyses, organ weights, histology or renal function tests (Chamorro et al., 1988[c]).

Table 9. Reproductive toxicity studies showing no adverse effects of *Spirulina maxima**

Animals	Dose	Duration	Test parameters	Reference
Rats	*S. maxima* 10, 20, and 30% in the diet	Males-9 wk; Females-from 2 wk before mating to 3 wk after delivery	Fertility and pregnancy; the number of live or dead pups at birth, survival rate, or weaning rate.	Salazar et al., 1996
Mice	*S. maxima* 10, 20, and 30% in the diet	Males-9 wk; Females-from 2 wk before mating to gestation day 18	Fertility and pregnancy; the number of *corpora lutea*, total implantations, or number of live or dead fetuses. The number of resorptions.	Chamorro et al., 1997
Mice	Unidentified species, up to 500 mg/kg BW/d	Gestating females; up to gestation d 17	$CdCl_2$-induced teratogenicity; external, visceral and skeletal abnormalities of fetus	Romeo-Manilla et al., 2008
Mice	*S. maxima* 10, 20, and 30%	The algae was given on gestation day 7-14, 1-14, and 1-21.	Teratogenicity; maternal and fetal wt. and no embryo resorptions of fetuses	Chamorro et al., 1989[b]
Mice	*S. maxima* 30%	19-20 d of pregnancy and 0-4 postnatal days	Peri- and post-natal development; pregnancy and litter, BW, and survival rate	Chamorro et al., 1997
Rats	*S. maxima* 10, 20, and 30% in the diet	Over three generations	Multigenerational; fertility, gestation, size of litters, or fetus viability, and subchronic toxicity parameters of F3 generation	Chamorro et al., 1985,
Rats	*S.maxima*; 10, 20, and 30% in diet	13 wk for F3 generation	NOAEL, 30% in the diet. No toxic effects on food consumption, BW, hematology, clinical chemistry, urine analyses, organ weights, histology or renal function tests	Chamorro et al., 1988[c]

*Modified from Chamorro et al., 2008.

1.5. Genetic Toxicity

Effects of Spirulina on genotoxicity have been studied in rats and mice as well as in bacteria (Table 10). There are reports that *Spirulina platensis* and other types of Spirulina exerted antigenotoxic effects in various animal models (Qishen et al., 1989; Zhang et al., 2001). For example, *Spirulina platensis* (12 mg/kg) increased the level of red cells, white cells, and hemoglobin in blood and nucleated cells in bone marrow in dogs (Qishen et al., 1989). Polysaccharide of Spirulina platensis reduced hematopoietic damage induced by injection of cyclophosphamide (CTX) and 6^0Co-gamma irradiation in mice and dogs (Zhang et al., 2001). *Spirulina platensis* (30, 60 mg/kg) increased the concentration of the white cells in blood and nucleated cells and DNA in bone marrow in mice but had no effects on red cells and hemoglobin.

Other types of Spirulina also showed protection against genetoxicity induced by cisplatin and urethane (Prekumar et al., 2004) as well as by cyclophosphamide and/or mitomycin-C (Table 10; Chamorro et al., 2006; Prekumar et al., 2001). Models have been developed that allow for the detection of potential damage induced in germ cells at a specific stage of development, to be expressed through weekly mating, or of the potential damage induced in germ cells at various stages of development, to be expressed during the first week of mating, respectively. In both cases, *Spirulina maxima* showed no genotoxicity in mice and rats (Chamorro and Salazar, 1989, 1995; Salazar and Chamorro, 1990). Urine of animals fed Spirulina for 4 mo showed no mutagenicity when the Ames test was conducted with five strains of *S. typhimurium* and *Schizosaccharomyces pombe* (Bizzi et al., 1980).

Table 10. Antitoxic effects of Spirulina against genotoxicity*

Animals	Dose and duration	Measurements	Reference
Mice and dogs	Polysaccharide of *S. platensis*, 30 and 60 mg/kg, 21 d	Gamma-radiation-induced damage in hematopoietic system; The level of the white cells in blood and nucleated cells, as well as DNA in bone marrow in mice. The level of the red cells, white cells, and hemoglobin in blood as well as nucleated cells in bone marrow in dogs.	Zhang et al., 2001
Mice	*S. platensis* extract (1-5 mg/kg) given orally	The micronucleus test; mouse bone marrow polychromatic erythrocytes against gamma-radiation injury.	Qishen et al., 1989
Mice	*S. fusiformis* (250, 500, and 1000 mg/kg)	The micronucleus; inhibition of genotoxicity and reduced lipid peroxidation induced by cisplatin and urethane	Prekumar et al., 2004
Mice	*S. fusiformis* (250, 500, and 1,000 mg/kg)	Micronucleus test; chromosomal damage and lipid peroxidation induced by cyclophosphamide and mitomycin-C.	Prekumar et al., 2001
Mice	*S. maxima* given orally (200, 400, or 800 mg/kg)	Antimutagenic effect against cyclophosphamide, evaluated by the dominant-lethal test.	Chamorro et al., 2006

*Modified from Chamorro et al., 2008.

1.6. Antioxidant and Antitoxic Effects of *Spirulina*

Numerous studies have demonstrated antioxidant effects of *Spirulina platensis* (Bhat and Madyastha, 2001; Chu et al., 2010; Dartsch, 2008; Kim et al., 2010). Other types of Spirulina also showed antioxidant effects (unidentified species, *S. maxima, or S. fusformis* (Kuhad et al., 2006; Thaakur and Jyothi, 2007; Wu et al., 2005).

Antioxidant properties of *Spirulina platensis* may partly contribute to antitoxic effects against nephrotoxicity, cardiotoxicity, ovary toxicity, and metal-induced toxicity as well as antiviral and immune-enhancing effects (Tables 11-13; Avdagic et al. 2008; Grawish et al., 2010; Karadeniz et al., 2008; Karaka et al., 2007; Khan et al., 2005, 2006; Kumar et al., 2009; Lu et al., 2010; Simsek et al., 2009; Zaccaro et al., 2004). No studies reported any side effects of *Spirulina platensis*; in these studies, dosages up to 1,000 mg/kg BW/d were not associated with any adverse effects.

Other types of Spirulina also showed similar antitoxic effects (Ble-Castillo et al., 2002; Gonzalez de Rivera et al., 1993; Haque and Gilani, 2005; Kuhad et al., 2006; Mohan et al., 2006; Torres-Duran et al., 1998; Vadiraja et al., 1998). C-phycocyanin, a potent antioxidant which has the ability to scavenge free radicals, is known as an active component in Spirulina (Bhat and Madyastha, 2000; Deng and Chow, 2010; Hsiao et al., 2005).

Table 11. Antitoxic effects of *Spirulina platensis*

Animals	Methods	Measurements	Reference
Rat	1,000 mg/kg, orally, 9 d	Gentamicin-induced acute tubular necrosis	Avdagic et al. 2008
Rat	1,000 mg/kg, orally, 7 d	Gentamicin-induced nephrotoxicity	Karadeniz et al., 2008
Rat	500 mg/kg, 17 d	Cyclosporine-induced nephrotoxicity, as measured by MDA rise in plasma and kidney tissues, isometric vacuolization and interstitial widening.	Khan et al., 2006
Hamster	*S.platensis* extract, 10 mg/d, 24 wk	DMBA-induced carcinogenicity measured by pathological alterations (immunohistochemical)	Grawish et al., 2010
Mice	500 mg/kg BW/d, 7.5 wk	Doxorubicin-induced cardiotoxicity, measured by lipid peroxidation, antioxidant enzymes, total antioxidant activity, and histopathology of heart	Khan et al., 2005
Mice	2,6, 9% (or up to 1,350 mg/kg BW/d) in diet, 1 wk	Acetaminophen-induced liver injuries, examined by hepatic malonaldehyde, serum conc. of GOT, GPT, and IL-8	Lu et al., 2010
Rat	300 mg/kg	Ovary toxicity; lead-induced alterations in the number of mast cells in the cortex and medulla of rat ovaries during the oestrus cycle.	Karaka et al., 2007
Mice	*S.platensis*, 28 d	Hematological damage induced by ultraviolet (UVC)-irradiation	Zaccaro et al., 2004
Rat	400 mg/kg BW/d. Up to 45 d	Normalization of collagen-induced arthritis, examined by the joint histopathology, serum chemistry, lipid profile, and lipid peroxidation.	Kumar et al., 2009
Rat, female	300 mg/kg BW/d, 30 d	Cadmium- or lead-induced toxicity, examined by hematological values	Simsek et al., 2009

Table 12. Effects of *Spirulina platensis* on immune function

Animals	Dose and duration	Measurements	Reference
Chicken	0.5, 1.0, or 2.0% in diet, 14, 35, 42 d	Macrophage function	Al-Betshan et al., 2001
In vitro	Methanolic extract of S. platensis	Release of histamine from mast cells	Price et al., 2002
Mice	10 or 20% (or up to 30,000 mg/kg BW/d) in diet, 46 or 67 d	Antibody production; macrophage functions, phagocytes and IL-1 production	Hayashi et al., 1994

Table 13. Antiviral activities of *Spirulina platensis*

Animals	Dose	Measurements	Reference
Rat	*S. platensis* extract, 0.3-1.2 ug/ml	Antiviral activity, measured by HIV-1 replication in human T cell line	Ayehunie et al., 1998
In vitro	*S. platensis* extract, 20-30 ul of 100 mg/ml extract	Gram positive and gram negative bacteria and *Candida albicans* ATCC 10239	Ozdemir et al., 2004

2. Human Feeding Programs for Nutritional Rehabilitation

Spirulina has been used as a health food supplement for malnourished children and adults in clinical studies since the early 1970's (Deng et al., 2010; Habib et al., 2008; Seshadriet al., 1993). Because of its unusual high nutritional values, several Spirulina feeding programs were administered in various countries. Examples include the following:

1. The Intergovernmental Institution for the use of Micro-algae Spirulina Against Malnutrition (IIMSAM) was launched in the mid-1970's to promote Spirulina as a high nutritional food to fight against starvation and malnutrition in the world (Habib et al., 2008).
2. India had a one year feeding program of Spirulina (1 g/d) with 5,000 pre-school children. A Spiulina-supplemented diet reduced the occurrence of "Bitof's spot", a symptom of vitamin A deficiency, from 80% to 10% (Seshadriet al., 1993).

3. Due to its concentrated nutrition, Spirulina was recommended for astronauts by National Aeronautics and Space Administration (Deng et al., 2010).

3. Human Clinical Studies

Recent human clinical trials have demonstrated various health benefits in malnourished young children, healthy volunteers, or subjects with non-insulin dependent diabetes mellitus (NIDDM), allergic rhinitis, or hypercholesterolemia with no side effects (Table 14). Studies on nutrition rehabilitation support various nutrition feeding programs involving Spirulina. For example, Simpore et al. (2009) assessed the impact of an elementary integrator composed of Spiruline (*Spirulina platensis,* 10 g/d, corresponding to 1,666 mg/kg BW/d) and Misola (millet, soja, and peanut) on the nutritional status of undernourished children. Five hundred fifty (550) undernourished children (severe marasma, 57 marasma of medium severity, and 38 kwashiorkor plus marasma) of less than 5 years old were given one of 4 types of diets for 8 wk: Misola (731 ± 7 kcal/day), Spiruline plus traditional meals (748 ± 6 kcal/day), Spiruline plus Misola (767 ± 5 kcal/day), and traditional meals (722 ± 8 kcal/day; the control group). Weight gain was observed in all the groups; 20 g/d in the Misola group, 25 g/d in the Spiruline plus traditional meals group, 34 g/d in the Misola plus Spiruline group and 15 g/d in the control group. However, Spiruline plus Misola appeared to have synergic effects on the nutrition rehabilitation better than traditional meals. (i.e., the simple addition of protein and energy intake). These pre/post differences within groups were statistically significant considering the differences in the nutritional status changes across the groups, but this difference was less significant in the control group. No side effects were reported related to daily consumption of 10 g (1,666 mg/kg BW) *Spirulina platensis.*

Another Spirulina supplementation study (8 wk) with undernourished HIV-infected and HIV-negative children resulted in similar outcomes (Simpore et al., 2005). Rehabilitation with Spirulina platensis showed an average weight gain of 15 g/d (corresponding to 2,479 mg/kg BW/d) and 25 g/d (corresponding to 4,132 mg/kg BW/d) in HIV-infected and HIV-negative children, respectively. The level of anemia decreased during the study in all children, but recuperation was less efficient among HIV-infected children: 81.8% in HIV-negative and 63.6% in HIV-infected. These studies support the historic usage of Spirulina for improving the nutritional status of malnourished population.

Other demonstrated benefits of *Spirulina platensis* include hypocholesterolemic and hypoglycemic effects, antioxidant and immunity effects, and reduction of skeletal muscle damage during exercise (Table 14; Baicus and Baicus, 2007; Cingi et al., 2008; Kalafati et al., 2009; Kim and Kim, 2005; Lee et al., 2008; Lobner et al., 2008; Lu et al., 2006; Park and Kim, 2005; Park et al., 2008; Selmi et al., 2011). No studies reported side effects of *Spirulina platensis.*

Table 14. Human clinical studies demonstrating beneficial or no adverse effects of *Spirulina platensis*

Subjects	Dose, g/d	Duration	Measurement endpoints	Ref
550 undernourished children aged 6-60 mo (median age 15.3 mo)	10 g/d (corresponding to ave. of 1,666 mg/kg BW/d)	8 wk	Nutritional rehabilitation including correction of BW loss	Simpore et al., 2006
166 malnourished children, HIV-infected and HIV-negative	15 or 25 g/d (corresponding to ave. of 2,479 or 4,132 mg/kg BW/d)	8 wk	Nutritional rehabilitation; BW gain and hematological parameters including anemia	Simpore et al., 2005
12 healthy elderly (mildly hyper-cholesterolemic and normocholesterolemic subjects)	7.5 g/d	24 wk	Serum lipid profiles	Park and Kim, 2005
51 elderly women with hypercholesterolemia	7.5 g/d	8 wk	Serum lipid profiles and oxidized LDL, Apolipoprotein B, and IL-6 concentration, and IL-6 production by peripheral blood lymphocyte.	Kim and Kim, 2005
37 patients with NIDDM	8 g/d	12 wk	Serum lipid profiles, blood pressure, IL-6 levels, and plasma malondialdehyde	Lee et al., 2008
78 healthy elderly volunteers aged 60-87 y	8 g	16 wk	Serum lipid profiles, IL-2, IL-6, superoxide dismutase	Park et al., 2008
8 volunteers	3 g	4 wk	Improvement of idiopathic chronic fatigue	Baicus and Baicus, 2007
150 allergic rhinitis patients aged 19-49 y	2 g/d	26 wk	Improvement of allergic rhinitis	Cingi et al., 2008
11 healthy males	Polysaccharide extracts from S. platensis, 200 mg/d	56 d	Adaptive immune response	Lobner et al., 2008
9 moderately trained males	6 g/d	4 wk	Ergogenic and antioxidant effects during exercise	Kalafati et al., 2009
16 college students	7.5 g/d	3 wk	Skeletal muscle damage under exercise-induced oxidative stress	Lu et al., 2006
40 elderly aged 50 or older	3.0 g/d	12 wk	Immune functions (indoleamine 2,3-dioxygenase activity) and serum hematology	Selmi et al., 2011

Dosages=dosages of dried *Spirulina platensis* powder unless noted otherwise.

Also, human clinical trials on other types of Spirulina (unidentified species, *S. maxima*, or *S. fusiformis*) reported similar benefits with no side effects (Table 15; Becker et al., 1986; Branger et al., 2003; Cingi et al., 2008; Ferreira-Hermosillo et al., 2010; Juarez-Oropeza et al., 2009; Kamalpreet et al., 2008; Kaur et al., 2008; Mani et al., 2000; Mathew et al., 1995; Nielson et al., 2010; Nikaya et al., 1988; Parikh et al., 2008; Ramamoorthy and Premakumari, 1996; Samuels et al., 2002; Torres-Duran et al., 2007).

Taken together, human studies showed that *Spirulina platensis* is safe for human consumption and daily consumption of approximately 4,132 mg/kg BW/d did not result in any adverse effects, even in infants and young children. Also, no member of the Genus *Spirulina* is known to be toxic.

Table 15. Human clinical studies demonstrating beneficial or no adverse effects of other types of *Spirulina*

Subjects	Dose, g/d	Duration	Measurement endpoints	Ref
165 infants	Spirulina (unidentified species), 5 g/d (or 1,000 mg/kg BW/d)	3 mo	Infant malnutrition	Branger et al., 2003
19 overweight subjects	Spirulina (unidentified species), 7.2 g/d	2 wk	Weight loss	Becker et al., 1986
30 healthy volunteers (Male)	Spirulina (unidentified species), 4.2 g/d	4 or 8 wk	Serum lipid profiles	Nikaya et al., 1988
15 Patients with NIDDM	Spirulina (unidentified species) tablets, 2 g/d	2 mo	Blood sugar, lipid, and glycated serum protein levels	Mani et al., 2000
25 patients with NIDDM	SUNOVA spirulina (unidentified species) capsules, 2 g/d	2 mo	Serum lipid profiles and the level of apolipoprotein A1.	Parikh et al., 2008
60 patients with NIDDM aged 40-60 y	SUNOVA spirulina (unidentified species) capsules, 2 g/d	2 mo	Blood lipid and sugar profiles	Kaur et al., 2008
23 Patients with nephritic syndrome	Spirulina (unidentified species) capsules, 1 g/d	2 mo	Serum lipid profiles	Samuels et al., 2002
36 allergic rhinitis patients aged 18-55 y	Spirulina (unidentified species), 2 g/d	12 wk	Improvement of allergic rhinitis, as measured by cytokines	Cingi et al., 2008
12 healthy volunteers aged 26-69 y	Spirulina (unidentified species), 0.4 g/d	7 d	Killing K562 tumor cells by natural killer cell activity	Nielson et al., 2010
60 Patients with type 2 diabetes aged 40-60 y	SUNOVA Spirulina capsules(unidentified species), 1 or 2 g/d	2 mo	Serum lipid profiles and both fasting and postprandial blood glucose levels	Kamalpreet et al., 2008
36 Healthy volunteers	*S. maxima*, 4.5 g/d	6 wk	Serum lipid profiles and blood pressures	Torres-Duran et al., 2007
36 Healthy volunteers aged 18-65 y	*S. maxima*, 4.5 g/d	6 wk	Vascular reactivity	Juarez-Oropeza et al., 2009
30 Patients with ischaemic heart disease	*S. fusiformis*, 2 or 4 g/d	3 mo	Plasma lipid profiles	Ramamoorthy and Premakumari, 1996
60 Patients with oral leukoplakia	*S. fusiformis*, 1g/d	1 y	Reversing oral leukoplakia	Mathew et al., 1995
3 Patients with non-alcoholic fatty liver disease	*S. maxima,* 4.5 g/d	3 mo	Blood lipid profiles and alanine aminotransferase conc.	Ferreira-Hermosillo et al., 2010

Dosages=dosages of dried *Spirulina* powder unless noted otherwise.

4. **Allergy**

Rare cases of side-effects (gastrointestinal discomfort) may happen with excessive intakes (over 100 g/d).

5. **Potential contamination**

Anatoxin-a, a cyanotoxin with acute neurotoxicity, was detected in 3 of the 39 cyanobacterial samples (Rellan et al., 2009). It was thus recommended that quality control of cyanobacterial food supplements including *Spirulina* was required to avoid potential adverse effects in animals and humans.

IV. Conclusions

This GRAS determination for *Spirulina platensis* is based upon scientific procedures. There is abundant literature describing the composition and safety of *Spirulina platensis*. Also, numerous human and animal studies examined the health benefits of *Spirulina platensis*. There are no reports of safety concerns in any of the studies. RFI utilizes a HACCP-controlled manufacturing process and rigorously tests its final production batches to verify adherence to quality control specifications.

The information/data provided by RFI in this report and supplemented by the publicly available literature/toxicity data on *Spirulina platensis* provide a sufficient basis for an assessment of the safety of *Spirulina platensis* for the proposed use as an ingredient in foods and beverages, when prepared according to appropriate specifications and used according to GMP. Key findings are summarized here:

1. *Spirulina platensis* is well characterized and free from chemical and microbial contamination.
2. Manufacturing processes of *Spirulina platensis* have been safely used for many years in the food industry.
3. The safety and nutritional benefits of *Spirulina platensis* are well established by human clinical trials and animal studies. There are no indications of significant adverse effects related to *Spirulina platensis* consumption in the publicly available literature. *Spirulina platensis* and its active component, phycocyanin, are classified into the “practically nontoxic” category. Subchronic toxicity and reproductive toxicity studies showed that consumption of dried *Spirulina platensis* up to 1,200 mg/kg BW/d, the maximum level tested, had no adverse effects. Human studies showed that daily consumption of 4,132 mg/kg BW of *Spirulina platensis* did not result in any adverse effects even in infants and young children.
4. *Spirulina platensis* has a long history of safe use in foods. *Spirulina platensis* is of natural biological origin and has been widely consumed for its nutrient properties, without known detrimental effect.

Therefore, not only is the proposed use of *Spirulina platensis* in foods and beverages safe within the terms of the Federal Food, Drug, and Cosmetic Act (meeting the standard of reasonable certainty of no harm), but because of this consensus among experts, it is also GRAS.

V. Discussion of information inconsistent with GRAS determination

We are not aware of information that would be inconsistent with a finding that the proposed use of *Spirulina platensis* preparations in foods and beverages, meeting appropriate specifications and used according to GMP, is GRAS.

VI. Availability of Information

The data and information that serve as the basis for this GRAS Notification will be available for review and copying at reasonable times at the offices of:

RFI
300 Corporate Drive, Suite 14
Blouvelt, NY 10913
TEL: 800-962-7663

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