GR

ORIGINAL SUBMISSION

AIBMR
Life Sciences, Inc.

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

July 12, 2011

ATTN: Dr. Antonia Mattia, PhD

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

Dear Dr. Mattia,

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

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

Parry Nutraceuticals (the notifier), has determined that Certified Organic Spirulina is Generally Regarded as Safe (GRAS), consistent with section 201 (s) of the Federal Food, Drug and Cosmetic Act. This determination has been made based on scientific procedures, and includes reference to FDA GRAS notification No. 127, which was filed for Spirulina in 2003. Spirulina has a long history of human consumption and has been thoroughly researched in toxicological models, with no results prompting concern for safety. It has been recommended as a food for human consumption by governmental agencies. In summary, the use of Certified Organic Spirulina in the enclosed specified categories of food is exempt from the requirement of pre-market approval.

Yours sincerely,

(b) (6)

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

Submitted by the Notifier:

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

Prepared by the Agent of the Notifier:

AIBMR Life Sciences, Inc
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July 12th, 2011

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1. GRAS Exemption Claim

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

(b) (6) 29th Jun 2011

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

Date

(i) Name and Address of the Notifier

a. Notifier

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

b. Agent of the Notifier

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(ii) Common or Usual Name

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

(iii) Conditions of Use

Certified Organic Spirulina is intended for use at levels of 0.5–3 grams per serving, as an ingredient in the following food categories; *beverages and beverage bases* (nonalcoholic, including only special or spiced teas, soft drinks, coffee substitutes, and fruit and vegetable flavored gelatin drinks); *breakfast cereals* (including ready-to-eat and instant and regular hot cereals); *fresh fruits and fruit juices* (including only raw fruits, citrus, melons, and berries, and home-prepared "ades" and punches made therefrom); *frozen dairy desserts and mixes* (including ice cream, ice milks, sherbets, and other frozen dairy desserts and specialties); *grain products and pastas* (including macaroni and noodle products, rice dishes, and frozen multicourse meals, without meat or vegetables); *milk products* (including flavored milks and milk drinks, dry milks, toppings, snack dips, spreads, weight control milk beverages, and other milk origin products); *plant protein products* (including the National Academy of Sciences/National Research Council "reconstituted vegetable protein" category, and meat, poultry, and fish substitutes, analogs, and extender products made from plant proteins); *processed fruits and fruit juices* (including all commercially processed fruits, citrus, berries, and mixtures; salads, juices and juice punches, concentrates, dilutions, "ades", and drink substitutes made therefrom); *processed vegetables and vegetable juices* (including all commercially processed vegetables, vegetable dishes, frozen multicourse vegetable meals, and vegetable juices and blends) *snack foods* (including chips, pretzels, and other novelty snacks); *soft candy* (including candy bars, chocolates, fudge, mints, and other chewy or nougat candies); and *soups and soup mixes* (including commercially prepared meat, fish, poultry (at levels that fall within FDA jurisdiction), vegetable, and combination soups and soup mixes).

(iv) Basis for GRAS determination

Scientific procedures are the basis for this GRAS determination.

(v) Data/Information Availability Statement

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

2. Characterization

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

A. platensis is one of three commonly cultivated and investigated *Arthrospira* cyanobacterium species. The other two are *A. maxima* and *A. fusiformis.* All three are frequently referred to by the traditional names of *Spirulina platensis, Spirulina maxima,* and *Spirulina fusiformis.* Microscopically, they appear as blue-green filaments composed of cylindrical cells arranged in unbranched, helicoidal trichomes. The main morphological feature of the genus is the open left hand helix arrangement along the entire length of the multicellular trichomes. The slight differences between the species are mainly related to the architecture of the trichomes. For instance, *A. maxima's* trichomes, as compared to the trichomes of *A. platensis,* are often wider in diameter, attenuated at the ends, and less constricted at the crosswalls (Vonshak 1997).

However, different growth and stress conditions cause morphological elasticity variations among these species, causing confusion among the scientific community concerning species nomenclature. According to Tomaselli, in *Spirulina platensis (Arthrospira): Physiology, Cell-biology, and Biotechnology,* it remains uncertain whether *A. maxima* and *A. fusiformis* can be considered separate species from *A. platensis* (Vonshak 1997; Scheldeman *et al.* 1999). For this reason, toxicological studies using various strains and even species, such as *A. maxima,* are considered when determining safety profiles.

Arthrospira species have recently undergone reclassification. The most current and authoritative work in bacterial taxonomy uses a new classification system based on phylogenetic lines instead of morphology (Garrity 2005).

Chemical Composition

Certified Organic Spirulina is a fine, uniform powder, blue-green to green in color, with a mild odor and taste. It is unique in its chemical composition because of its high nutritional density; it contains a wide range and abundance of macro- and micronutrients, as well as phytochemicals. In terms of macronutrients, Certified Organic Spirulina contains high levels of protein (56–69%) with a

balanced amino acid profile, 15–25% carbohydrates (mainly glucose), and 5–6% lipids, mainly as polyunsaturated fatty acids with a high ratio of gamma-linolenic acid (GLA). The micronutrient composition features relatively high concentrations of B-vitamins, especially cyanocobalamin (0.05–0.20 mg/100g). Spirulina's phytonutrient composition includes phycocyanins (phycobiliproteins involved in light harvesting reactions), carotenes (including beta-carotene), chlorophyll, and xanthophylls. Batches of Certified Organic Spirulina are routinely assayed in Parry Nutraceuticals' Quality Assurance Lab for protein, moisture, total ash (minerals), bulk density, light filth, algal (microcystin) toxins and microbes in addition to the phytopigments listed above (see Table 3 in section 3 (iv) a., entitled specifications and batch analyses).

Organic Certification

In addition to USDA National Organic Program (NOP) organic standards, Parry Nutraceuticals cultivates Certified Organic Spirulina under a number of other major international organic standards: Naturland (Germany), ECOCERT certification (France), and OCIA -USA (Organic Crop Improvement Association). The product is also certified Kosher (Star K) and Halal.

Parry Nutraceuticals' Spirulina is certified by USP under the USP Ingredient Verification Program—meeting USP specifications for GMP, manufacturing, quality control documentation, and label claims. These accredited organizations are world leaders in the certification of organic products, and their certifications guarantee conformity and traceability to American, European, or other standards.

3. Manufacturing and Production

(i) Company Overview

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

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

(ii) Raw Materials

Along with the Spirulina inoculum, the raw materials used in the manufacturing of Certified Organic Spirulina include water, inoculation nutrients, and pond cultivation nutrients. The inoculation and pond cultivation nutrients, discussed below, consist of sea salt and organic nutrients of plant origin. The materials are purchased from approved vendors. All materials must arrive with supporting documents. Incoming raw materials are inspected, sampled, and stored in designated raw material storage houses. Random samples of 2% of the number of containers received are sent to Parry Nutraceuticals' in-house laboratory or approved outside laboratories where they are tested and verified by designated authorities.

Water

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

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

Inoculation Nutrients

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

The raw materials used for the initial inoculation stage are added to distilled water and the Spirulina inoculum. These materials constitute the inoculation medium, known as the Modified Zarrouk Medium. The nutrients for preparing the Modified Zarrouk Medium are listed in Table 1. The concentrations are per 1 liter distilled water.

Table 1. Modified Zarrouk Medium

Organic nitrogen source	5.0 g
Sea salt	1.0 g

Pond Cultivation Nutrients

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

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

(iii) Manufacturing Overview

Parry Nutraceuticals cultivation and production process is certified under ISO 9001 (quality management systems), ISO 14001 (environmental management systems), and ISO 22000 (food safety management systems). Quality systems have been established to ensure that all of the algal products produced meet the requirements established in food GMP (CFR part 110).

Parry Nutraceuticals' production process follows the open raceway pond design developed in the 1950's and widely used for the outdoor mass cultivation of photosynthetic microorganisms. This process involves indoor and then semi-outdoor cultivation of the Spirulina culture, followed by outdoor pond cultivation, harvesting, washing, drying, packing, warehousing and shipping. The entire production process is designed and carefully monitored to encourage *A. platensis* growth and prevent contamination.

Cultivation

The initial indoor cultivation involves growing Spirulina culture in sterile flasks under artificial lights in the prescribed organic medium, which consists of distilled water and the raw materials discussed previously. It is then progressively cultured and sub-cultured, first in flasks and then in larger plastic tubs. The outdoor cultivation involves the inoculation of tub cultures into a seed pond, followed by the progressive inoculation of other seed ponds until a certain level of biomass is met, at which time this culture is used to inoculate ½-acre production ponds. This organic production pond is then sub-cultured again into ponds earmarked for Spirulina production. The production ponds may contain

varying volumes of culture depending on the pond depth. The ponds are agitated by paddle wheels in order to facilitate light distribution and nutrient distribution, minimize self-shading due to buoyancy, and maintain a uniform temperature. Nutrients (as listed under Raw Materials), and soft water are added to the ponds, which are agitated daily.

Pond Maintenance and Monitoring

The maintenance and monitoring of the ponds involves daily culture medium testing (sample collection, pond depth maintenance, optical density, and pH), microscopy, chemical analysis, and meteorological notation (temperature, sunshine duration, and rainfall). After agitating for two hours, a 50 mL culture sample is collected daily from a marked place in all ponds. From the sample, optical density is measured using a spectrophotometer in order to estimate biomass density. (The timing of the culturing and sub-culturing process, in which the Spirulina culture is inoculated in a series of progressively larger tubs and ponds, is based on the optical density.) The pH is measured using a pH meter. Microscopy is also performed daily. A drop of culture from each pond is examined microscopically for contamination by other algae, rotifers, zooplanktons, motile flagellates, and precipitates. In addition, the health and size of the trichomes, which are *Arthrospira's* distinguishing morphological feature (and described in more detail earlier in this document), are determined daily via microscopy. Chemical analysis of the culture medium is done on an as-needed basis and includes testing for nitrogen, phosphorous, sulfur, bicarbonate, and carbonate using standard methods. Spirulina is selected for harvest based on the pond's biomass levels, the culture medium testing, and the microscopy results.

Harvesting and Packaging

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

(iv) Specifications, Batch Analysis and Quality Management

a. Specifications and Batch Analyses

Production consistency is tested in production lots. As shown in Table 3 below, four non-sequential batches were reasonably consistent and met the product specifications for physical/general composition, phytopigments, heavy metals, microbial analyses and absence of microcystin completely.

Table 3: Specifications and Batch Analysis of Organic Spirulina

Parameter	Specification	Method	Lot Numbers			
			PS-0424-VNK/10-11	PS-0425-VNK/10-11	PS-0531-VNK/10-11	PS-0532-VNK/10-11
Physical Properties/General Composition						
Protein (% dry wt)	56–69	AOAC 978.04 16th Edition	62.14	62.20	62.05	62.10
Moisture (% dry wt)	2.5–6.0	AOAC 934.01 16th Edition	4.20	4.15	4.2	4.15
Total Ash (Minerals) (% dry wt)	6.0–9.0	AOAC 930.05 16th Edition	6.99	7.02	6.90	7.10
Bulk Density (g/cc)	0.62–0.85	C.Vijayaraghavan (1995). A Practical handbook of physical pharmaceutics, 1995.	0.769	0.769	0.769	0.769
Phytopigments						
Total Carotenoids (mg/100 g dry wt)	400–650	Strickland and Parsons (1972). A practical Handbook of Seawater Analysis	425	434	426	420
Beta Carotene (mg/100 g dry wt)	150–250	Ranganna S. (1986). Handbook of analysis and QC for fruit & veg. Products.	162	165	164	162
Xanthophylls (mg/100 g dry wt)	250–470	In house method	263	269	262	258
Crude Phycocyanin (% dry wt)	15–19	Boussiba, S, Arch. Microbiol, 120:155 – 159, 1979	16.57	16.62	16.44	16.37
Chlorophyll-a (% dry wt)	1.23–1.67	Vonshak.A.1997. Spirulina platensis (Arthrospira) physiology, cell biology and biotechnology.	1.48	1.49	1.42	1.39
Total Pheophorbide (% dry wt)	≤ 0.12	A. Test method for Spirulina by JHFA, Environmental Food Number 99 (1981). B. Seward R. Brown. Absorption Coefficients of Chlorophyll Derivatives. J.Fish. Res. BdCanada 25 (3) 523 – 540, 1968	0.019	0.021	0.021	0.022
Existing Pheophorbide (% dry wt)	≤ 0.08		0.017	0.019	0.020	0.020
Light Filth (pieces/50g)	≤ 50	Richard Gorham. J. (1977). Training manual for Analytical Entomology in the food industry. FDA Tech. Bulletin No: 2	5	6	7	7
Heavy Metals						
Lead (ppm)	≤ 0.2	AOAC 18th Edition: 2006 by ICPMS	0.108	0.0938	0.137	0.135
Arsenic (ppm)	≤ 0.5		0.300	0.270	0.252	0.252
Cadmium (ppm)	≤ 0.2		<0.0100	0.0112	0.0176	0.0186
Mercury (ppm)	≤ 0.05		<0.0100	<0.0100	<0.0100	<0.0100

Microbials						
Standard Plate Count (cfu/g)	≤ 50,000	Bacteriological Analytical manual 8th Edn, AOAC, USFDA, 1995.	8000	8200	8500	8200
Yeast and Mold (cfu/g)	Not more than 100		30	25	30	30
Coloforms (Enterobacteriaceae) (/25g)	Negative		Negative	Negative	Negative	Negative
E. Coli (/25g)	Negative		Negative	Negative	Negative	Negative
Salmonella (/25g)	Negative		Negative	Negative	Negative	Negative
Staphylococci (/25g)	Negative		Negative	Negative	Negative	Negative
Algal Toxin						
Microcystin (ppb/3g—DL: 0.5 ppb)	Not Detectable	Lawrence Et Al,: Journal of AOAC International Vol. 84, No.4, 2001	Complies	Complies	Complies	Complies

b. Residual Pesticide and Other Contaminant Analysis

Routine analysis of Certified Organic Spirulina powder for pesticides, aflatoxins, ochratoxin A, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and organochlorine and organophosphorous pesticides is carried out once per year. A random sample is sent to accredited laboratories identified by Parry. The compounds tested for by SGS laboratory in the three batches reviewed for this notification (batch numbers PS-0004-CNK/11-12, PS-0005-CNK/11-12 and PS-0006-CNK/11-12) include:

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

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

c. Algal Toxins and Pheophorbides

To date, there is no report of any cyanobacterial toxins above specified limits in *Arthrospira* species, and their discovery is considered very unlikely to occur during monoculture of Spirulina in properly controlled and managed systems (Gershwin *et al.* 2008). It is true that some cyanobacteria can produce

hepatotoxins or neurotoxins. For example, *Microcystis aeruginosa* produces microcystins, which are potent hepatotoxins and probable tumor promoters. Health Canada surveyed *Aphanizomenon flos-aquae* blue-green algal products from Upper Klamath Lake in southern Oregon to determine levels of microcystins and detected microcystins in 85 of 87 samples tested, with 63 samples (72%) containing concentrations of > 1 μg/g, the regulatory limit established by the Oregon Health Division and the Oregon Department of Agriculture (Gilroy *et al.* 2000). Despite the fact that *A. platensis* is not considered a toxigenic cyanobacteria species and that cultivation occurs in controlled ponds rather than natural lakes, Parry routinely tests finished products for microcystins and **has never detected** this toxin.

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

d. Shelf-life Stability

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

Additionally, a real-time shelf-life study was performed, beginning July 2009. The ingredient was stored in a food grade aluminum pouch with N2 flushing, inside a box with stretch wrapping and tied with pop tape—stored at ambient temperature. The last measurement was performed on April 2011 (after 36 months). The sample will continue to be tested to determine the ultimate shelf-life. After 36 months, parameters including moisture, total carotenoids (mg/100g), beta carotene (mg/100 g), crude phycocyanin (%), chlorophyll a (%), standard plate microbial count and yeast and mold (cfu/g) still fell within specifications for the product. Xanthophyll levels (mg/100 g) decreased just slightly below specifications; measuring 249.8 mg/100 g after 12 months (specification 250–470 mg/100 g) and 243.9 mg/100 g after 36 months. This particular batch began with a xanthophyll level on the low end of the

specification at the baseline measurement (252.7 mg/100 g), and some analytical variation is expected during the testing process. Additionally, 100 g is much higher than the expected serving size; hence the slightly decreased measurement on this larger scale would reflect a minimal change at the serving size level.

4. Self-limiting Levels of Use

There are no specific self-limiting levels of use for Certified Organic Spirulina.

5. Safety Assessment

(i) Toxicology Studies

GRN 000127, which was filed by FDA without questions, presents a detailed discussion of animal and human safety studies establishing quality and safety standards for Spirulina. In animals, safety evaluations involving acute, subchronic, chronic, mutagenic, teratogenic, carcinogenic, and multiple generation effects have been conducted over the last three decades. Both short- and long-term toxicity evaluations from various institutions around the world using different Spirulina samples demonstrate absence of toxicity in animals. A large number of human studies that utilized Spirulina are reported in the literature, a number of which indirectly attest to its safety.

The majority of toxicology studies in the literature on Spirulina were recently reviewed by Spirulina researcher and toxicologist Dr. German Chamorro of the National School of Biological Sciences, National Polytechnic Institute, México (Gershwin *et al.* 2008). In the 1970s and 1980s, Dr. Chamorro led comprehensive Spirulina animal studies sponsored by the United Nations Industrial Development Organization (UNIDO). Subchronic toxicity, chronic toxicity, effects on reproduction and lactation, teratogenicity, and mutagenicity were studied (Chamorro-Cevallos 1980). The results of these studies, along with some additional toxicology studies performed over the last twenty-five years, are summarized here.

a. Sub-Chronic Oral Toxicity Studies

The objective of the UNIDO-sponsored subchronic study was to detect any possible toxicity at Spirulina levels beyond the 10% of the diet that had been used in a previous study by Till and Willems in 1971 (Chamorro-Cevallos 1980). Levels of 20% and 30% were used in order to detect any toxicity at these higher percentages. Wistar rats were fed a diet of 10, 20, and 30% Spirulina (species not identified) in place of soy for a period of 13 weeks. Two control groups consisted of a soy-based diet group and a group fed a commercial diet commonly used in the laboratory. The five total groups each had 10 male and 10 female rats. Chamorro concluded that Spirulina fed to Wistar rats for three months at levels of 10, 20, and 30% in the diet did not affect any of the parameters studied, including weight, behavior, appearance, food consumption, hematologic or urologic parameters, GOT, GPT, alkaline phosphatase, and macroscopic and histopathological organ changes. No statistically significant changes occurred in

any of the parameters with the exception of the relative weights of the seminal vesicles of animals treated with 20 and 30% Spirulina. Chamorro concluded that this was not of toxicological interest because there were no pathological findings during histopathological examination (Chamorro-Cevallos 1980).

Other subchronic studies using different feed preparations, administration times, and types of analyses have collectively found that Spirulina, even in high concentrations, produced no adverse effects (Gershwin *et al.* 2008). In a subchronic toxicity study in mice, *A. maxima* up to high feeding levels did not produce adverse effects after 13 weeks of treatment. Groups of 10 mice of each sex were given *S. maxima* in the diet at concentrations of 0 (control), 10, 20 or 30% for 13 weeks. The Spirulina had no effect on behavior, food and water intake, growth or survival. Terminal values in hematology and clinical chemistry did not reveal differences between treated and control groups. Post-mortem examination revealed no differences in gross or microscopic findings (Salazar *et al.* 1998).

GRN 000127 describes other independent feeding tests in France, Italy, Mexico, Japan, and India conducted during the 1970's and 1980's showing no undesirable results or toxic side effects.

b. Chronic Oral Toxicity Studies

The objective of the UNIDO-sponsored tests for chronic toxicity, including functional tests, was to study the effects of large-scale Spirulina administration on hematological parameters, kidney function, serum chemistry parameters, and the weight and histopathology of certain organs. As in the sub-chronic study, Wistar rats were fed a diet of 10, 20, and 30% Spirulina in place of soy for a period of 80 weeks. Two control groups consisted of a soy-based diet group and a group fed a commercial diet commonly used in the laboratory. Each of the five total groups had 20 male and 20 female rats. Chamorro concluded that Spirulina did not produce any toxic effects in any of the parameters studied – weight gain, hematological parameters, liver and kidney function, terminal serum chemistry, mortality, relative organ weights, or histopathological parameters including tissue lesions and tumor incidence (Chamorro-Cevallos 1980).

Other chronic oral toxicity tests performed on rats to assess the cumulative toxicity of Spirulina also indicate no long-term effects on normal physiological or biological processes. For example, Wistar rats fed Spirulina at the maximum protein portion (14.25%) for 75 weeks showed no obvious signs of toxicity. Throughout the 18 months, the male animals fed the Spirulina diet showed weight increase comparable to the control animals fed a casein-based diet; the females fed Spirulina showed a slight decrease in weight up to the 30th week. Weight increases were comparable and a normal frequency of tumors occurred. No obvious signs of toxicity were observed. The authors concluded there was no evidence of toxicity related to the use of Spirulina as the sole source of protein in the rat (Boudene *et al.* 1975).

Other chronic toxicity studies were cited in Chamorro's recent review, including a 100-day study by Bourges et al in 1971, in which Chamorro reports that rats tolerated well a Spirulina-rich diet with a final concentration of 36% and 48%

protein. According to Chamorro, no histological abnormalities were found in several organs examined (Gershwin *et al.* 2008).

A 6-month oral toxicity study was performed on C57BL/6J mice using *Spirulina plantensis* in the diet at 0, 2.5 and 5% (n=8). Throughout the study, there were no signs of illness or behavioral changes in the mice, nor were there differences in body weight gain. Plasma AST levels were increased 2-fold in control male and female mice compared to baseline; this parameter was significantly lower in *S. platensis*-treated male mice after six months as compared to controls, and there was a trend toward a decrease in female mice as well. One hypothesis as to why this occurred is that the Spirulina may have prevented age-related tissue damage. There were no significant differences in liver histopathology compared to controls (Yang *et al.* 2011).

c. Reproductive and Developmental Toxicity Study

To detect any consequences of feeding Spirulina to successive generations, an UNIDO-sponsored multigenerational study on reproduction and lactation was spread over a two-year period (three generations in approximately two years) and was completed in the last generation by a conventional sub-chronic toxicity study (Chamorro-Cevallos 1980). Wistar rats were divided into five groups of five males and five females each, and the groups were fed the same diets as for the chronic toxicity study. Fertility, gestation, lactation, and viability indices were recorded. For the 13-week sub-chronic part of the reproduction study, 10 males and 10 females were randomly selected from the F3b generation and general condition, weight increase, consumption and conversion efficiency, hematology, serum and urine analyses, and weight and histopathological examination of the organs were performed.

In the first generation, the fertility index was similar in all groups, and no negative effects of Spirulina in regard to terminations were observed. No modifications were observed in the viability or lactation indices of the litters. Mean weights of the litters matched the controls. Results corresponding to the second mating of this generation were reduced in relation to results of the first matings but were similar to controls, and the litter weights of this generation were similar to the controls. The lactation index for this group was slightly reduced compared to the first mating, but the litter growth during weaning was the same for the Spirulina and control groups. The results of the second mating of the F1b generation and the F2b generation showed no variations between the groups. Overall, no effects on fertility, litter size, or mortality were observed.

The results of the 13-week subchronic study performed on the third generation groups found significant differences in the male 20% Spirulina group, and in the female control group. Significant differences were also noted in the weights of the hearts, kidneys, and seminal vesicles of the males in the treatment groups and in the weights of the lungs and spleens of some females in the treatment groups. The differences were not dose-dependent and were not accompanied by pathological differences that could be attributed to Spirulina toxicity. Macroscopic examination revealed hydronephrosis in all groups, but no histopathologically examined lesions were attributed to Spirulina.

In other reproductive toxicity studies undertaken since Chamorro's and mentioned in his recent review, no developmental abnormalities have been observed at any time between zygote formation and postnatal maturation in Spirulina-fed rats, mice or hamsters (Chamorro-Cevallos 1980). In one additional study, *A. maxima* consumption had no effects on reproduction and peri- and postnatal development. At levels of 0, 10, 20 and 30% *A. maxima* incorporated into the diet, there was no reduction in body weight gain in males or females and no deaths or clinical signs of toxicity. Treatment was not associated with any adverse effect on any measure of reproductive performance, including male and female fertility and duration of gestation (Salazar *et al.* 1996).

The objective of the UNIDO-sponsored teratogenicity studies was to detect any spirulina-related embryonic resorptions or fetal malformations (Chamorro-Cevallos 1980). Wistar rats, CD-1 mice, and Dorado hamsters born to mothers fed 10, 20, and 30% Spirulina (species not identified) diets were used, along with two groups of controls as in the previous UNIDO-sponsored experiments. The Spirulina was fed to the treatment groups over three different periods during gestation. After the pregnant animals were weighed and sacrificed, fetuses of the sacrificed animals were counted, weighed, and examined for internal (visceral and skeletal) or external malformations; uterine wall implantations were counted to determine embryonic resorptions. From the data, a mean teratogenic index was calculated using the average percentages of control and treated animals affected. Some isolated cases of statistical significance occurred in the mean weight of the fetus, the number of implantations per fertile female, and the number of fetuses per pregnant female, but they did not show any relationship to the level of Spirulina fed to the animals. Chamorro's overall conclusion was that Spirulina does not cause gestational changes indicated by malformations, anomalies, or resorptions (Gershwin *et al.* 2008).

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

(ii) Additional Scientific Studies

While Spirulina has been consumed for hundreds of years in the diet, a number of human clinical trials performed in recent years further support safety of oral administration of Spirulina by confirming the absence of adverse effects. GRN 000127 presents a detailed discussion of various human studies that used Spirulina as the test article. Some additional human studies reporting no adverse effects are summarized here:

- A randomized, double-blind, placebo-controlled cross-over study of Spirulina in overweight adults was conducted by the Institut für Chemische Pflanzenphysiologie der Universität in Tübingen, Germany. The study consisted of 15 overweight patients with a mean age of 35.8 years and a mean BMI of 30.5 ± 5.2. Intake of 2.8 g Spirulina three times per day (8.4 grams total per day) for 4 weeks was associated with no adverse effects on blood pressure, heart rate, CBC, blood chemistry panel, kidney function, enzyme activities, or physical symptoms (Becker *et al.* 1986).
- A government-sponsored one-year feeding program with 5,000 rural pre-school children in India showed a decrease in Bitot's spots (a symptom of Vitamin A deficiency) from 80% to 10% after consumption of 1 g Spirulina per day for at least 150 days. The Spirulina was incorporated into noodles sweetened with sugar to preserve the beta-carotene. Called "Spiru-Om", it was well accepted by the children. The study was conducted by the Shri Amm Murugappa Chettiar Research Center in Madras, India.
- To evaluate the effects of Spirulina, sixteen males and 20 females, all healthy adults, orally consumed 4.5 grams Spirulina per day (3 tablets of 0.5 grams every 8 hours) for six weeks. At the beginning of the study and every week, fasting blood samples were taken and glucose, TAG, TC, HDL-C, and AST levels were determined to assess the potential hepatotoxic effects of treatment. No changes were observed in AST and glucose values throughout the experimental period (35 ± 18 UI/L and 85 ± 13 mg/dL respectively. No adverse effects were reported and the study authors reported that safety of oral administration was demonstrated (Torres-Duran *et al.* 2007).
- Thirty men with cardiovascular disease risk factors were fed Spirulina in addition to their normal diet. Group A consumed 4.2 grams daily for eight weeks. Group B consumed Spirulina for four weeks and were observed for another four weeks. Fasting blood samples were collected and WBC, GOT, GPT, LDH, gamma-GTP, alkaline phosphatase, uric acid, BUN, and creatine were measured at weeks 0, 2, 4, 6, and 8. No significant changes occurred in any of these parameters. No adverse effects were reported by any of the subjects and no problems were found on clinical examination (Nakaya *et al.* 1988).
- Seventy-eight subjects, aged 60–87 years, were given either 8 grams per day of Spirulina or placebo for four months in a randomized double blind study. The authors concluded that Spirulina is a suitable functional food for the elderly, and consumption resulted in favorable effects on blood lipids, immune variables and antioxidant capacity (Park *et al.* 2008).
- Children were fed 10–15 grams per day as a dietary supplement mixed with millet, water and spices, resulting in recovery from malnourishment within several weeks {Habib, 2008 #62859}.
- A search of clinicaltrials.gov outlines studies that evaluate oral consumption of 5 to 19 grams of Spirulina daily in various populations, suggesting that these levels are considered safe for use in those populations (trial numbers NCT00680277, NCT01141777, NCT01084382, NCT01195077).

(iii) History of Consumption

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

Commercial Spirulina production began in the 1970's. Today, *A. platensis* is one of the most commercialized micro algae, being cultivated for mass production for use in human food, animal feed, and colorimetric industry (Kim *et al.* 2007). It has been sold both in the United States and around the world since the late 1970s as a food product and dietary supplement. More than 3,000 tons of *A. platensis* are produced annually worldwide, and the majority is used for health food products and animal feed additives (Eriksen 2008). Some of the products found currently in the marketplace are listed below in section 5(v).

(iv) Previous Sales and Reported Adverse Events

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

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

(v) Similar Products in the Marketplace

Spirulina is commercially available in a number of food and dietary supplement products as exemplified in Table 4 below:

Table 4: Commercially Available Food Products Containing *Spirulina*

Manufacturer	Product	Description	Label Claim
Betty Lou's, Inc. (USA)	Spirulina Ginseng Nut Butter Balls	Energy bar (ball)	1000 mg Spirulina
Raw Indulgence (USA)	Raw Revolution Organic Live Food Bars, Spirulina and Cashew	Raw food bar	Amount not listed
Lydia's Organics (USA)	Spirulina Bar	Raw food bar	Amount not listed
Freeland Foods (USA)	Spirulina Energy Bar	Raw food bar	Amount not listed
Rédei (Hungary)	Organic Spirulina spelt wheat pasta	Dried pasta	Amount not listed
Agisko (UK)	Spirulina pasta	Dried pasta	Amount not listed
K. Rogers Food Industries Sdn. Bhd. (Malaysia)	Spirulina and Cereal Butter Cookies & Spirulina Almond Cookies	Cookies	Amount not listed
Nature's Plus (USA)	SPIRU-TEIN® Bars, Wafers, Shakes, Ready-to-Drink	Protein bars, Protein powders, Protein wafers, Protein drinks	Amount not listed
Odwalla (USA)	Superfood	Green drink	1500 mg Spirulina
Odwalla (USA)	Superfood ™	Energy bar	500 mg Spirulina
Bolthouse Farms (USA)	Green Goodness	Green drink	Amount not listed
Naked Juice (USA)	Green Machine	Green drink	1300 mg Spirulina
Sambazon (USA)	Supergreens Revolution	Green drink	Amount not listed
Nutraceutical Sciences Institute	Spirulina	Powder to be added to water or juice	Suggested use: take 7000 mg spirulina one or more times daily
Earthrise	Spirulina Natural	Powder to be added to water or juice	Suggested use: take 3000 mg 1–2 times daily

(vi) Current Regulatory Status

An *Arthrospira platensis (A. platensis)* powder was the subject of a previous GRAS determination, submitted on behalf of Cyanotech Corporation and Earthrise Nutritionals, Inc. On the basis of scientific procedures, these two companies determined their spray-dried Spirulina powder is GRAS when used as an ingredient in foods such as bars, powdered nutritional drink mixes, popcorn, and as a condiment in salads and pasta, at levels ranging from 0.5 to 3 grams per serving size. FDA responded on October 6, 2003, having no questions regarding the notifiers' conclusion that Spirulina is GRAS under the intended conditions.

Since Parry Nutraceuticals' Certified Organic Spirulina is bioidentical to the subject of the previous notification, and because Parry Nutraceuticals follows the same raceway pond method of manufacturing, this GRAS determination incorporates by reference GRN 000127.

The Convention for the Use of Food Micro-Algae, Intergovernmental Institutional Spirulina Program (CISRI-ISP/ IIMSAM) works to promote the use of Spirulina against severe malnutrition. It has been established through two international agreements that are recognized in the UN Treaty Series. IIMSAM is accredited as a Permanent Observer Mission with the United Nations Economic and Social Council. It was also recommended as one of the primary foods during long-term space missions by both the National Aeronautics and Space Administration (NASA) and the European Space Agency (ESA) (Deng *et al.* 2010) (Habib *et al.* 2008).

Clinical studies in India and Africa have shown that, when Spirulina is used as a food complement, there is a significant response in the improved nutritional status of undernourished children (Simpore *et al.* 2006). The European Commission's Humanitarian Aid department (ECHO) funds supplemental feeding programs for about 65,000 Sri Lankan war refugees. The project funds *A. platensis* farming as the main food source of a low budget, high nutrition diet.

Antenna Technology is a Swiss-based organization composed of scientists and researchers working on issues of malnutrition by introducing Spirulina as a tool to fight child malnutrition. Antenna's publication, *The Nutritional Aspects of Spirulina* cites a study at Hôpital Bichat, France, of malnourished children and adults who were given doses of 80–90 grams Spirulina per day. Absorption of Spirulina proteins was found to be good, and despite these very large doses, no noteworthy increase in blood uric acid was demonstrated. The original publication verifying this reference could not be obtained (Falquet 1996).

Shri AMM Murugappa Chettiar Research Centre (MCRC), Chennai, India is a non-Governmental Voluntary Research Organization established in 1977. The MCRC trains rural people especially women to grow Spirulina for nutrition and income generation. They conducted the 18-month trial with 5,000 pre-school children listed under "Clinical Trials" above.

The Food and Agriculture Organization of the United Nations considers Spirulina to have significantly high macro- and micronutrient content, and discusses its important use as human food in its 2008 document entitled "A Review on Culture, Production and Use of Spirulina as a Food for Humans and Feeds for Domestic Animals and Fish" (Habib *et al.* 2008).

(vii) Information that may appear to be Inconsistent with GRAS Determination

Spirulina is considered safe for human consumption based on its long history of use as a food source, as well as numerous animal and human studies that demonstrate no safety concerns. There have been rare adverse incidences that have coincided with consumption of Spirulina supplements, although Spirulina causation has not been proven. A single case study was reported concerning a 28-year-old man who developed rhabdomyolysis after taking Spirulina for one month. He reported that he was not taking other supplements or medications and had no other risk factors (Mazokopakis *et al.* 2008). In contrast, a small clinical study showed potential preventive effects of exercise-induced skeletal damage (Lu *et al.* 2006). Another single case study was published of a 52-year-old Japanese man who showed signs of hepatotoxicity after taking Spirulina, although he was also taking three other medications. The signs resolved after discontinuation of the Spirulina and all medications (Iwasa *et al.* 2002). Lastly, a single case study reported an allergic reaction to Spirulina (specifically the phycocyanin component) by a 14-year old boy (Petrus *et al.* 2010). The reaction was confirmed by a positive prick test and oral challenge.

6. Intended Use

(i) Categories of Food

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

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

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

> (3) Beverages and beverage bases, nonalcoholic, including only special or spiced teas, soft drinks, coffee substitutes, and fruit and vegetable flavored gelatin drinks.
>
> (4) Breakfast cereals, including ready-to-eat and instant and regular hot cereals.

(16) Fresh fruits and fruit juices, including only raw fruits, citrus, melons, and berries, and home-prepared "ades" and punches made therefrom.

(20) Frozen dairy desserts and mixes, including ice cream, ice milks, sherbets, and other frozen dairy desserts and specialties.

(23) Grain products and pastas, including macaroni and noodle products, rice dishes, and frozen multicourse meals, without meat or vegetables.

(31) Milk products, including flavored milks and milk drinks, dry milks, toppings, snack dips, spreads, weight control milk beverages, and other milk origin products.

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

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

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

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

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

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

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

(ii) Estimated Daily Intake (EDI)—Exposure

In FDA GRAS notification No. 127 (submitted by Cyanotech Corporation and Earthrise Nutritionals, Inc.), which FDA filed with no questions, the Spirulina addition level was stated as 0.5 to 3 grams per serving. A high-end consumer was estimated to potentially consume 6 grams of Spirulina per day; a medium consumer was estimated to consume 3 grams per day, and a low-end consumer was estimated to consume a maximum of 12 grams per month. The Spirulina in the present GRAS notification is the same species of Spirulina as in GRAS notification 127, and uses a similar manufacturing process. Cyanotech Corporation and Earthrise Nutritionals, Inc. stated heavy metal specifications in their notification; < 0.05 ppm for mercury, < 0.5 ppm for cadmium, < 1.0 ppm for lead, and < 1.0 ppm for arsenic. The heavy metal specifications of Parry Nutraceutical's Organic Spirulina are the same and/or more conservative as compared to those in the Cyanotech and Earthrise notification, and are as follows; < 0.05 ppm for mercury, < 0.2 ppm for cadmium, < 0.2 ppm for lead, and < 0.5 ppm for arsenic.

Organizations around the world generally encourage consumption of Spirulina rather than limit it, especially in populations that are malnourished {Habib, 2008 #62859}. To calculate an estimated daily intake (estimated exposure) for Certified Organic Spirulina, a USDA Nutrition Insights article (a publication of the USDA Center for Nutrition Policy and Promotion)—Insight 20 October 2000 (Basiotis *et al.* 2000) is referenced. According to this publication, males aged 51 or greater consume the greatest number of servings of food per day. They consume an average of 18.2 total servings of food per day from the following food groups: grains, fruits, vegetables, milk, meat and other (fats, oils, sweets). According to the same publication, the smallest number of total daily servings of food is that consumed by females aged 19–24, who consume 12.5 total servings per day. Because Certified Organic Spirulina is intended for use in foods categories that span all of these food groups, it is reasonable to consider this data when calculating the estimated daily intake of Spirulina.

For the purpose of this GRAS notification, using the above referenced USDA data, the estimated number of total food servings per day for a high-end consumer would be 18.2 (males aged 51 or greater). The number of total servings consumed by females aged 19–24 (12.5 servings per day) was used for calculations to represent a low-end user. If one quarter of a person's daily food intake contained Certified Organic Spirulina at the same addition levels as specified in FDA GRAS No. 127 (0.5–3 grams per serving) and using the USDA figures for the minimum and maximum number of servings of food consumed in the US, the resulting daily exposure would range from 2.28–13.7 g/day.

7. General Recognition

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

8. Basis for the GRAS Determination

Based on an independent and collective critical evaluation of the data and information described above, the Expert Panel, qualified by scientific training and experience to evaluate the safety of substances added to food, concluded that Parry Nutraceutical's Certified Organic Spirulina, when produced according to Good Manufacturing Practice and meeting the specifications presented in this notification, is generally recognized as safe for its intended use based on scientific procedures, and is hence exempt from the requirement of premarket approval. The previous FDA GRAS notification No. 127 for Spirulina, which was filed by FDA without question, was taken into account for this determination, as were the numerous toxicological studies performed on Spirulina. A plethora of publications in the public domain, including human and animal studies, demonstrate that there is common knowledge and consensus among qualified experts that Spirulina is safe for its intended use. The safety and quality control data that are the basis of the safety evaluation are corroborated by the long and extensive history of safe consumption of Spirulina by humans worldwide.

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