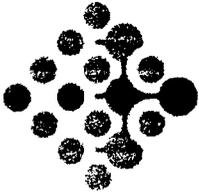


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Original Submission

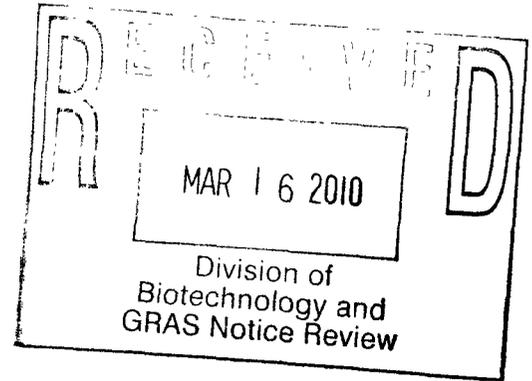
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SENT VIA FEDEX

March 12, 2010

Robert L. Martin, Ph.D.
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food And Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835



Re: GRAS Notice for an Algal oil (*Chlorella*) ingredient

Dear Dr. Martin:

In accordance with proposed 21 CFR §170.36 [Notice of a claim for exemption based on a Generally Recognized As Safe (GRAS) determination] published in the *Federal Register* [62 FR 18938 (17 April 1997)], I am submitting in triplicate, as the notifier [Solazyme, Inc. (Solazyme), 561 Eccles Avenue, South, San Francisco, CA 94080, USA], a Notice of the determination, on the basis of scientific procedures, that Solazyme's Algal oil (*Chlorella*) ingredient, as defined in the enclosed documents, is GRAS under specific conditions of use as a food ingredient, and therefore, is exempt from the premarket approval requirements of the *Federal, Food, Drug and Cosmetic Act*. Information setting forth the basis for the GRAS determination, which includes a comprehensive summary of the data available and reviewed by an independent panel of experts in support of the safety of the Algal oil (*Chlorella*) ingredient under the intended conditions of use, also are enclosed.

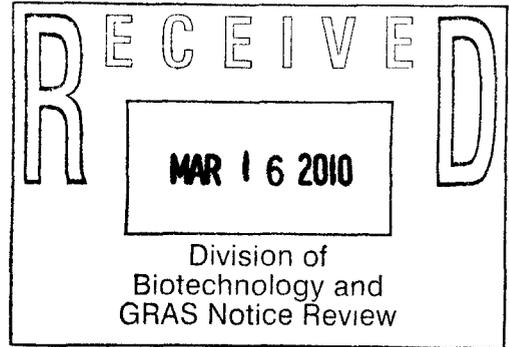
I trust that the enclosed Notice is acceptable. Should you have any questions or concerns regarding this GRAS Notice, please do not hesitate to contact me at any point during the review process so that we may provide a response in a timely manner.

Sincerely, (b) (6)

Anthony Day Ph.D.
Vice President of Research & Development

Enclosure

000004



ALGAL OIL (*CHLORELLA*) GRAS NOTICE

Prepared for:

Robert L. Martin, Ph.D.
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835

Prepared by:

Solazyme, Inc
561 Eccles Avenue, South
San Francisco, CA 94080
USA

March 4, 2010

000005

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Algal Oil (*Chlorella*) Notification

I. GRAS Exemption Claim

I.A. Claim of Exemption from the Requirement for Premarket Approval Pursuant to Proposed 21 CFR §170.36(c)(1) [62 FR 18938 (17 April 1997) (U.S. FDA, 1997)]

As defined herein, Algal oil (*Chlorella*) produced from the dried biomass of *Chlorella protothecoides* S106 has been determined by Solazyme Inc. to be Generally Recognized as Safe (GRAS), consistent with Section 201(s) of the *Federal Food, Drug, and Cosmetic Act* (U.S. FDA, 2009a). This determination is based on scientific procedures as described in the following sections, and on the consensus opinion of an independent panel of experts¹ qualified by scientific training and expertise to evaluate the safety of Algal oil (*Chlorella*) under the conditions of its intended use in food. Therefore, the use of Solazyme's Algal oil (*Chlorella*) in food as described below is exempt from the requirement of premarket approval.

Signed,

(b) (6)

Anthony Day Ph.D.
Vice President of Research & Development
Solazyme, Inc
561 Eccles Avenue, South
San Francisco, CA 94080
USA

3-10-10
Date

I.B. Name and Address of Notifier

Solazyme, Inc
561 Eccles Avenue, South
San Francisco, CA 94080
USA

I.C. Common Name of the Notified Substance

Algal oil (*Chlorella*)

¹ The Panel consisted of the below-signed qualified scientific experts: Professor Joseph F. Borzelleca, Ph.D. (Virginia Commonwealth University School of Medicine), Professor Eric A. Johnson, Sc.D. (University of Wisconsin – Madison), and Professor Gary M. Williams, M.D. (New York Medical College). A copy of the Expert Panel Consensus Statement is located in Appendix C and is titled "Expert Panel Consensus Statement Regarding the Generally Recognized as Safe (GRAS) Status of *Chlorella* Oil for Use in Food". Note that Solazyme's Algal oil (*Chlorella*) – the ingredient that is the subject of this Notification – is referred to as *Chlorella* oil throughout the Expert Panel Consensus Statement in Appendix C.

Algal Oil (*Chlorella*) Notification

I.D. Conditions of Intended Use in Food

Solazyme intends to market Algal oil (*Chlorella*) as a food ingredient in the United States under the proposed food uses at use levels ranging from approximately 1.8 to 26%, with the exception of frying, cooking, and specialty oils at a use-level of 100%, as described in Table A-1 (Appendix A).

I.E. Basis for the GRAS Determination

Pursuant to 21 CFR § 170.30, Algal oil (*Chlorella*) has been determined by Solazyme to be GRAS on the basis of scientific procedures (U.S. FDA, 2009b). This GRAS determination is based on data generally available in the public domain pertaining to the safety of Algal oil (*Chlorella*) for use in food, as discussed herein, and on a consensus among a panel of experts who are qualified by scientific training and experience to evaluate the safety of Algal oil (*Chlorella*) as a component of food.

I.F. Availability of Information

The data and information that serve as the basis for this GRAS Notification will be sent to the U.S. Food and Drug Administration (FDA) upon request, or will be available for review and copying at reasonable times at the offices of:

Solazyme, Inc
561 Eccles Avenue, South
San Francisco, CA 94080
USA

Should the FDA have any questions or additional information requests regarding this notification, Solazyme, Inc will supply these data and information.

II. Detailed Information About the Identity of the Substance

II.A. Identity

Solazyme's Algal oil (*Chlorella*) is produced from the dried biomass of *Chlorella protothecoides* S106. *C. protothecoides* S106 is a species within the *Chlorella* genus

Common or Usual Name: Algal oil (*Chlorella*)

Chemical Name: *Chlorella protothecoides* S106 oil

Chemical Abstracts Service (CAS) Number: Not applicable

Empirical Formula: Not Applicable

Molecular Weight: Not Applicable

Algal Oil (*Chlorella*) Notification

Chemical and Physical Characteristics: *Chlorella* species are eukaryotic, unicellular, non-motile freshwater green algae that belong to the Division Chlorophyta (Kay, 1991). *Chlorella* cells have hemicellulotic cell walls and are spherical with a diameter ranging from 2 to 10 µm (Kay, 1991; Becker, 2007).

II.B. Method of Manufacture

Algal oil (*Chlorella*) is produced from the dried biomass of a pure culture of *C. protothecoides* S106. All raw materials used in the growth medium, fermentation process, production of oil, and all antioxidants used in the manufacturing process are suitable food-grade materials and are used in accordance with applicable U.S. federal regulations as described in Table II.B-1 below.

Table II.B-1 Raw Materials in the Growth Medium, Fermentation Process, and Production of Oil and Antioxidants in the Manufacture of Algal Oil (<i>Chlorella</i>)		
Material	Use	Regulatory Status
Growth Medium		
Dipotassium Phosphate (K ₂ HPO ₄)	Component	GRAS as a sequestrant under 21 CFR § 182.6285 when used in accordance with cGMP
Monosodium Phosphate (NaH ₂ PO ₄)	Component	Direct food substance affirmed as GRAS as an emulsifier and emulsifier salt, a lubricant and release agent, and as a surface-active agent when used at levels that do not exceed cGMP (21 CFR § 184.1521)
Yeast Extract	Component	Permitted as a non-standardized food under 21 CFR § 102.22
Magnesium Sulfate (MgSO ₄ ·7H ₂ O)	Component	Direct food substance affirmed as GRAS for use as a flavor enhancer, nutrient supplement, or processing aid with no limitation other than cGMP (21 CFR § 184.1443)
Ferric citrate	Component	Direct food additive affirmed as GRAS as a nutrient supplement with no limitation other than cGMP (21 CFR § 184.1298)
Industrol® 204	Component	Approved defoaming agent under 21 CFR § 173.340 when used in accordance with 21 CFR § 172.808
Cupric Sulfate, Pentahydrate (CuSO ₄ 6H ₂ O)	Component	Direct food additive affirmed as GRAS as a nutrient supplement or processing aid with no limitation other than cGMP (21 CFR § 184.1261)
Cobalt Chloride, Hexahydrate (CoCl ₂ 6H ₂ O)	Component	GRAS as a trace mineral when added to animal feeds (21 CFR § 582.80)
Boric Acid (H ₃ BO ₃)	Component	Approved as an indirect food additive for paper and paperboard products in contact with dry food according to 21 CFR § 176.180
Zinc Sulfate, Heptahydrate (ZnSO ₄ 7H ₂ O)	Component	GRAS as a nutrient under 21 CFR § 182.8997
Manganese Sulfate, Monohydrate (MnSO ₄ H ₂ O)	Component	Affirmed as GRAS as a direct food substance with no limitation other than cGMP (21 CFR § 184.1461)
Sodium Molybdate, Dihydrate (Na ₂ MoO ₄ 2H ₂ O)	Component	High purity (99.5 to 103.0%) and complies with the specifications of the American Chemical Society (ACS).

Algal Oil (*Chlorella*) Notification

Table II.B-1 Raw Materials in the Growth Medium, Fermentation Process, and Production of Oil and Antioxidants in the Manufacture of Algal Oil (<i>Chlorella</i>)		
Material	Use	Regulatory Status
Nickel (II) Chloride, 6-Hydrate (NiCl ₂ 6H ₂ O)	Component	Elemental nickel is affirmed as GRAS as a direct food substance for use as a catalyst in the hydrogenation of fats and oils under 21 CFR § 184.1537 with no other limitations than cGMP
Citric Acid	Component	Direct food substance affirmed as GRAS with no limitations other than cGMP (21 CFR § 184.1033)
Calcium Chloride (CaCl ₂)	Component	Direct food substance affirmed as GRAS as an anticaking agent, antimicrobial agent, curing or pickling agent, firming agent, flavor enhancer, humectant, nutrient supplement, pH control agent, stabilizer and thickener, surface-active agent, texturizer, and as a processing aid at levels not to exceed cGMP (21 CFR § 184.1193)
Thiamine Hydrochloride	Component	Direct food substance affirmed as GRAS with no limitations other than cGMP (21 CFR § 184.1875)
Biotin Powder	Component	GRAS for use as a nutrient when used in accordance with cGMP (21 CFR § 182.8159)
Cyanocobalamin (vitamin B12)	Component	Direct food additive that is affirmed as GRAS under 21 CFR § 184.1945
Calcium Pantothenate, Powder (D-Pantothenic Acid Hemicalcium Salt)	Component	GRAS and permitted to be used in foods at levels not to exceed cGMP (21 CFR § 184.1212)
Aminobenzoic Acid	Component	This ingredient is food-grade and complies with USP specifications.
Ammonium Sulfate	Component	Direct food substance affirmed as GRAS for use as a dough strengthener, firming agent, and a processing aid when used at levels not to exceed cGMP (21 CFR § 184.1143)
Inositol	Component	Direct food substance affirmed as GRAS as a nutrient supplement and when used in special dietary foods with no limitations other than cGMP (21 CFR § 184.1370)
Choline Chloride	Component	GRAS as a nutrient when used in accordance with cGMP (21 CFR § 182.8252)
Fermentation Process		
Glycerol	Cryoprotectant to the master seed culture	Indirect food additive intended for repeated use in contact with food (21 CFR § 177.2420)
Ammonium Hydroxide (NH ₄ OH)	Control pH	Affirmed as GRAS (21 CFR § 184.1139) as a pH control agent with no limitation other than cGMP
Potassium Hydroxide (KOH)	Control pH	Affirmed as GRAS (21 CFR § 184.1631) as a pH control agent with no limitation other than cGMP
Glucose as 95DE Corn Syrup or Equivalent	Prevent carbon starvation	Corn syrup, meeting the specification defined in 21 CFR § 168.120 (b), is a direct food substance affirmed as GRAS for use in food with no limitation other than cGMP
Sucrose	Prevent carbon starvation	Direct food substance affirmed as GRAS (21 CFR § 184.1854) to be used in foods with no limitation other than cGMP

Algal Oil (*Chlorella*) Notification

Table II.B-1 Raw Materials in the Growth Medium, Fermentation Process, and Production of Oil and Antioxidants in the Manufacture of Algal Oil (<i>Chlorella</i>)		
Material	Use	Regulatory Status
Oil Production		
Isohexane (2-Methylpentane)	Extract the dried <i>Chlorella</i> powder or remove residual oil	Hexane is a secondary direct food additive permitted in food for human consumption as a solvent, lubricant, release agent, or a related substance under 21 CFR § 173.270
Isopropanol	Solvent to remove residual oil	Secondary food additive permitted for direct addition to food for human consumption in spice oleoresins, in lemon oil, or in hops extract (21 CFR § 173.240)
Tonsil® Bleaching Earth	Refining step	Affirmed as GRAS for use as food processing aids when used in accordance with cGMP (21 CFR § 184.1155)
Antioxidants		
Fortium® Brand MTD10 Liquid Antioxidant (consists of canola oil and natural mixed tocopherols)	Antioxidant, stabilize the oil	Canola oil is affirmed as GRAS as a direct food substance (21 CFR § 184.1555) and tocopherols are GRAS as chemical preservatives under 21 CFR § 182.3890
En-Hance™ Brand A103S Liquid Antioxidant (composed of 32% mono- and diglycerides, 30% soybean oil, 20% tertiary butyl hydroquinone, 15% propylene glycol, and 3% citric acid)	Antioxidant, stabilize the oil	Tertiary butylhydroquinone is a food additive permitted for direct addition to food for human consumption as an antioxidant at levels such that the total antioxidant content of the food does not exceed 0.02% of the fat content of the food (21 CFR § 172.185)
Fortium® Brand RPT40 Liquid Antioxidant (Rosemary extract, mixed tocopherols, soybean oil, sunflower lecithin, and ascorbyl palmitate are contained in this antioxidant)	Antioxidant, stabilize the oil	Tocopherols are GRAS as chemical preservatives (21 CFR § 182.3890)
Grindox™ 497 Kosher Antioxidant (vitamin C palmitate, natural tocopherols, and lecithin with soybean oil)	Antioxidant, stabilize the oil	Vitamin C palmitate and tocopherols are GRAS as chemical preservatives (21 CFR § 182.3149 and 21 CFR § 182.3890, respectively). Lecithin is a direct food additive affirmed as GRAS with no limitation other than cGMP (21 CFR § 184.1400)

cGMP = current good manufacturing practice; FDA = Food and Drug Regulations; GRAS = generally recognized as safe; USP = United States Pharmacopeia. CFR = U.S. Code of Federal Regulations (U.S. FDA, 2009b)

The source strain for Algal oil (*Chlorella*) is *C. protothecoides* strain number UTEX 250, which was obtained from the University of Texas Culture collection and assigned Solazyme strain number S106. Master and working cell banks were prepared from the culture, and molecular genotyping conducted using 3 samples from each of the master and working cell banks demonstrated 100% identity between the 6 chromosomal footprints and 100% identity between their 23S ribosomal deoxyribonucleic acid (DNA) sequences. The 6 23S ribosomal DNA sequences also demonstrated 100% identity to the 23S reference sequence for the original S106 isolate.

Algal Oil (*Chlorella*) Notification

The manufacturing process for Solazyme's Algal oil (*Chlorella*) begins with the fermentation of the *C. protothecoides* source organism. A pure, clonally isolated culture of *C. protothecoides* is initially used to prepare a master seed bank from which working seed vials are prepared. As described above, the Master and Working seed banks were characterized by molecular methods to show that they were genetically identical. For a production lot, a cryopreserved working seed vial is thawed and used to inoculate a flask, which is transferred into larger flasks at mid-log phase, and then to standard, industrial seed fermentors. Throughout the fermentation process, pH, temperature, and agitation and aeration rates are controlled, and glucose or sucrose and nutrient feeds are added. Using controlled fermentation conditions, either a low- or high-lipid containing *C. protothecoides* algal biomass can be produced. In this process, *C. protothecoides* is first cultivated so as to produce a low lipid content, and lipid production is induced by limiting inorganic nitrogen during the latter part of the fermentation. Following completion of growth, the fermentation broth is harvested, concentrated, and dried. The oil is extracted from the dried biomass using mechanical or hexane extraction and may be further refined, bleached, and deodorized. If food-grade anti-oxidants are used, they are added to the oil prior to the packaging (*i.e.*, after extraction, or after refining, bleaching, and deodorizing of the oil).

II.C. Specifications for Food Grade Material

Algal oil (*Chlorella*) is produced in accordance with current good manufacturing practice (cGMP) and food grade chemical specifications have been established for the final product by Solazyme to ensure a consistent, safe product. The chemical specifications for Algal oil (*Chlorella*) are presented in Table II.C-1. Algal oil (*Chlorella*) contains $\leq 1\%$ free fatty acids, $\leq 2.0\%$ unsaponifiable matter, $\leq 1.0\%$ moisture and volatiles, and has an iodine value between 80 and 110 and a peroxide value of ≤ 5.0 meq/kg fat. Analysis of 1 lot of the crude Algal oil (*Chlorella*) and 3 non-consecutive lots of the refined, bleached, and deodorized Algal oil (*Chlorella*) indicate that the manufacturing process produces products that are consistent with the specifications, including limits set for residual solvents and heavy metals. The complete analyses of these batches are presented in Table B-1 (Appendix B). The main fatty acids present in Algal oil (*Chlorella*) are C18:1 (as oleic acid), C18:2 and C16:0. All analytical procedures are conducted using standard validated methodologies [*i.e.*, Association of Official Analytical Chemists (AOAC), American Oil Chemists' Society (AOCS)].

Algal Oil (*Chlorella*) Notification

Table II.C-1 Chemical Specifications for Algal Oil (<i>Chlorella</i>)		
Specification Parameter	Specification	Method
Free Fatty Acid	≤1%	AOCS Ca 5a-40
Unsaponifiable Matter	≤2.0%	AOCS Ca 6a-40
Moisture & Volatiles or Karl Fisher Moisture	≤1.0%	AOCS Ca 2c-25; AOCS Ca 2d-25 (vacuum) AOCS Ca 2e-84
Iodine Value	80 -110	Adaptation of AOAC Tg 1a-64
Peroxide Value	≤5.0 meq/kg	AOCS Cd 8-53
Residual hexane	<1.0 ppm	AOCS Ca 3b-87
Heavy Metals		
Lead	<0.5 ppm	AOAC Cd 17-01 (modified)
Arsenic	<0.2 ppm	AOAC Cd 17-01 (modified)
Mercury	<0.2 ppm	AOAC Cd 17-01 (modified)
Cadmium	<0.1 ppm	AOAC Cd 17-01 (modified)
Chromium	<2 ppm	AOAC Cd 17-01 (modified)

II.D. Stability of Algal Oil (*Chlorella*)

The oxidative rancidity of the oil in samples of Algal high-lipid flour (*Chlorella*) was used to assess the stability of Algal oil (*Chlorella*). The samples were prepared with 1 of 3 different antioxidants added at different levels and were assessed under ambient (23°C) and accelerated (40°C) storage conditions. Samples were packaged in individual, sealed 150 gram foil packets and a new package was opened at each test period. Peroxide values and alkenal values were monitored using the AOAC approved Saftest® System, manufactured by MP Biomedicals (Solon, OH). Algal oil (*Chlorella*) and Algal flour (*Chlorella*) are manufactured the same way, with the exception of the final steps where oil is extracted from the flour. The interim results are presented in Table II.D-1 below. At Day 148, all samples tested were below the cut off value for peroxide value of 5.0 meq/kg fat. A 12-month shelf life has been designed for Algal oil (*Chlorella*). Formal accelerated stability studies are ongoing.

Algal Oil (*Chlorella*) Notification

Treatment	Use-Level (ppm)	Peroxide Values (meq/kg fat) ¹						Malonaldehydes (mg/kg)					
		Day 0	Day 34	Day 62	Day 97	Day 118	Day 148	Day 0	Day 34	Day 62	Day 97	Day 118	Day 148
Untreated Control	0	0.08	0.36	0.05	0	4.28	4.85	0	0	0	21.65	0.64	0
ENHANCE A103S	250	0.11	0.10	0	0	0.85	4.04	0	0	0	13.67	0.29	0
	500	0.11	0.05	0	0	0.32	2.18	0	0	0	12.62	0.32	0
FORTIUM MTD10	375	0.08	0.44	0.04	0	4.60	4.90	0	0	0	15.16	0.65	0.04
FORTIUM RPT40	125	0.09	0.45	0.04	0	4.32	4.64	0	0	0.31	23.62	0.59	0.09
	250	0.13	0.13	0.01	0	3.73	4.79	0	0	0	11.40	0.62	0.07

¹ Based on 50% fat

III. Self-Limiting Levels of Use

Under the intended conditions of use of Algal oil (*Chlorella*), no self-limiting use levels are expected.

IV. Basis for GRAS Determination

The determination that Algal oil (*Chlorella*) is GRAS is on the basis of scientific procedures. The safety of Algal oil (*Chlorella*) under the intended conditions of use is based on an estimate of the probable consumption of the ingredient as calculated using the most recent publicly-available survey of U.S. food consumption in conjunction with data on vegetable oil consumption in the U.S., the results of a published product specific preclinical toxicity study on the source material [Algal high-lipid flour (*Chlorella*)], the compositional similarity of *C. protothecoides* to other *Chlorella* species such as *C. pyrenoidosa* and *C. vulgaris*, and published scientific data demonstrating that *Chlorella* sp. have low oral toxicity in animals and are well-tolerated by humans. Analyses of the chemical composition, amino acid composition, fatty acid composition, and of the genomic sequence of various species of *Chlorella* indicate that the *Chlorella* species are highly similar, supporting the basis for the bridging of both toxicological and clinical studies conducted with differing *Chlorella* species to support the safety of *C. protothecoides*.

These data were reviewed by a Panel of Experts, qualified by scientific training and experience to evaluate the safety of Algal oil (*Chlorella*) as a food ingredient, who concluded that the aforementioned proposed uses of Algal oil (*Chlorella*) are safe and suitable and would be GRAS based on scientific procedures (see Appendix C for a copy of the Expert Panel Consensus Statement) and that other qualified experts would concur with these conclusions. It also is Solazyme's opinion that other qualified and competent scientists reviewing the same publicly available toxicological and safety information would reach the same conclusion. A summary of these data is presented herein.

IV.A. Current Regulatory Status and History of Use

Chlorella has been consumed in the diet for centuries in countries such as Japan, China, and Korea. Over the last 50 years the production and consumption of *Chlorella* has increased following construction of manufacturing facilities in the U.S. and Taiwan (Ravishankar *et al.*, 2006) when it was discovered that large-scale culture production was technically feasible. As a dietary supplement, *Chlorella* has been widely consumed in Japan, Taiwan, and Australia in pill, capsule, and powder form for over 25 years.

Although not as popular as in Japan, a number of *Chlorella* dietary supplement products are available in the U.S., including *Chlorella regularis* Vcaps, manufactured by New Chapter, *Chlorella* tablets manufactured by Nature's Way, and *Chlorella* from Yaeyama tablets, manufactured by Source Naturals, all of which are available online from the Vitamin Shoppe, a U.S.-based specialty retailer of nutritional products (The Vitamin Shoppe, 2009). However, no official New Dietary Ingredient (NDI) Notification to the FDA was identified for these products. *Chlorella* is included in the United Natural Products Alliance (UNPA) (nee Utah Natural Products Alliance) "old" dietary ingredients guidance list, which contains dietary ingredients that were on the market prior to the implementation of *Dietary Supplement Health and Education Act of 1994* (DSHEA, 1994). Thus, *Chlorella* is sold and in the market place on the basis that it was marketed prior to the implementation of DSHEA (1994). *Chlorella* is available in capsule, tablet, or powder forms at dosages ranging from 200 to 500 mg/dosage unit (PDRNS, 2001) with recommended dosages up to 10 g per day.

IV.B. Estimated Intake of Algal Oil (*Chlorella*)

Algal oil (*Chlorella*) is intended to be used as an ingredient in baked goods and baking mixes, beverages (alcoholic), beverages and beverage bases, confections and frostings, fats and oils, frozen dairy desserts and mixes, gelatins, puddings, and fillings, grain products and pastas, milk products, nut and nut products, processed fruits and fruit juices, snack foods, soft candy, soups and soup mixes, and sweet sauces, toppings, and syrups at use-levels ranging from approximately 1.8 to 26%, with the exception of frying, cooking, and specialty oils at a use-level of 100%. The complete list of food-uses and use-levels is provided in Appendix A. The consumption of Algal oil (*Chlorella*) from all intended food uses was estimated using the National Center for Health Statistics' (NCHS) National Health and Nutrition Examination Surveys (NHANES) for the years 2005-2006 (NHANES 2005-2006) (CDC, 2006, 2009), which provide the most up to date data for evaluating food use and food consumption patterns in the U.S. Food codes representative of each intended food use were chosen from the NHANES 2005-2006 and were grouped in food-use categories according to Title 21, Section § 170.3 of the Code of Federal Regulations (U.S. FDA, 2009b).

Approximately 96.5% of the total U.S. population was identified as potential consumers of Algal oil (*Chlorella*) from the proposed food-uses (8,132 actual users identified). A high percentage of

Algal Oil (*Chlorella*) Notification

users were identified in all individual population groups (99.2 to 99.8%), with the exception of the infant population group (with 73.6% users). Consumption of these types of foods by the total U.S. population resulted in an estimated mean all-user intake of Algal oil (*Chlorella*) of 20.1 g/person/day on an absolute basis or 0.35 g/kg body weight/day on a body weight basis (Tables IV.B-1 and IV.B-2). The 90th percentile all-user intake of Algal oil (*Chlorella*) from all proposed food-uses by the total population was observed to be 39.4 g/person/day, or 0.73 g/kg body weight/day on a body weight basis.

Table IV.B-1 Summary of the Estimated Daily Intake of Algal Oil (*Chlorella*) from All Proposed Food-Uses in the U.S. by Population Group (2005-2006 NHANES Data)

Population Group	Age (years)	Percent Users	Actual # of Users	All-Person Consumption (g)		All-User Consumption (g)	
				Mean	90 th Percentile	Mean	90 th Percentile
Infants	0 to 2	73.6	723	8.2	20.2	10.3	22.7
Children	3 to 11	99.8	1,443	16.7	30.1	16.7	30.1
Female Teenagers	12 to 19	99.3	988	17.3	32.7	17.5	32.7
Male Teenagers	12 to 19	99.3	934	22.6	44.8	22.7	44.8
Female Adults	20 and up	99.8	2,148	19.5	39.1	19.6	39.1
Male Adults	20 and up	99.2	1,896	22.8	45.9	23.0	46.1
Total Population	All Ages	96.5	8,132	19.8	39.3	20.1	39.4

Table IV.B-2 Summary of the Estimated Daily Per Kilogram Body Weight Intake of Algal Oil (*Chlorella*) from All Proposed Food-Uses in the U.S. by Population Group (2005-2006 NHANES Data)

Population Group	Age (years)	Percent Users	Actual # of Users	All-Person Consumption (g/kg bw)		All-User Consumption (g/kg bw)	
				Mean	90 th Percentile	Mean	90 th Percentile
Infants	0 to 2	73.6	723	0.68	1.67	0.86	1.83
Children	3 to 11	99.8	1,443	0.64	1.23	0.64	1.23
Female Teenagers	12 to 19	99.3	988	0.30	0.57	0.30	0.57
Male Teenagers	12 to 19	99.3	934	0.36	0.74	0.36	0.76
Female Adults	20 and up	99.8	2,148	0.28	0.57	0.28	0.58
Male Adults	20 and up	99.2	1,896	0.27	0.52	0.27	0.52
Total Population	All Ages	96.5	8,132	0.34	0.73	0.35	0.73

Although Algal oil (*Chlorella*) is intended for use in all food categories indicated, it is highly unlikely that Algal oil (*Chlorella*) will be used in all food categories and all food-uses simultaneously due to the numerous other vegetable oils currently available on the market. In a

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more realistic scenario, Algal oil (*Chlorella*) is expected to achieve levels of use similar to those of olive, canola, sunflower, or peanut oil. Thus, to put the estimated intake of Algal oil (*Chlorella*) into perspective, the daily *per capita* consumption of other common vegetable oils is presented below in Table IV.B-3. As is summarized in the table, the daily *per capita* consumption of olive, sunflowerseed, groundnut (peanut), cottonseed, palm kernel, coconut, maize germ, and canola oil ranges from 0.14 g/person/day for palm kernel oil to 11.7 g/person/day for canola oil. Following soybean oil, the next most commonly consumed vegetable oil has a daily *per capita* intake that is approximately half the total population estimated mean all-user intake of Algal oil (*Chlorella*) and approximately 3 times lower than the total population estimated 90th percentile all-user intake of Algal oil (*Chlorella*). Therefore, it is expected that the estimated intakes for Algal oil (*Chlorella*) are substantial over-estimates of the anticipated actual consumption and that the actual levels of consumption will be more similar to the levels of olive, peanut, or sunflower oils.

Table IV.B-3 Estimated Daily <i>Per Capita</i> Consumption of Commonly Consumed Vegetable Oil in the United States		
Vegetable Oil	Daily <i>Per Capita</i> Consumption (g/person/day)	Source
Olive Oil	2.36 ¹	USDA, 2009a
	1.92 ²	FAOSTAT, 2003
Sunflowerseed Oil	0.25 ²	FAOSTAT, 2003
Groundnut Oil	1.07 ²	FAOSTAT, 2003
Cottonseed Oil	1.92 ²	FAOSTAT, 2003
Palmkernel Oil	0.14 ²	FAOSTAT, 2003
Coconut Oil	0.74 ²	FAOSTAT, 2003
Maize Germ Oil	4.00 ²	FAOSTAT, 2003
Canola Oil	11.7 ¹	USDA, 2009a
Soybean Oil	59.4 ²	FAOSTAT, 2003

¹ Data from 2007

² Data from 2003

Solazyme has noted that the methodology used to estimate the consumption of Algal oil (*Chlorella*) described above is generally considered to result in 'worst case' estimates of exposure as a result of several conservative assumptions made in the consumption estimates. For example, it is often assumed that all food products within a food category contain the ingredient at the maximum specified level of use. In addition, it is well established that the length of a dietary survey affects the estimated consumption of individual users. Short-term surveys, such as the typical 2- or 3-day dietary surveys, overestimate the consumption of food products that are consumed relatively infrequently. Thus, the estimated intakes reported in Tables IV.B-1 and IV.B-2 above are considered to be substantial over-estimates of the actual expected intake of Algal oil (*Chlorella*) in the U.S. population.

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Solazyme also has noted that although relatively high intake estimates of 22.7 g Algal oil (*Chlorella*)/day [1.83 g Algal oil (*Chlorella*)/kg body weight/day] were obtained in infants on an all-user basis from all proposed food-uses of Algal oil (*Chlorella*) at the 90th percentile, it should be stressed that the specified food-uses for Algal oil (*Chlorella*) are not intended to be marketed to infants, nor does Solazyme intend on marketing or formulating their products for consumption by children under 2 years of age. Thus, the actual infant consumption of Algal oil (*Chlorella*)-containing food products is expected to be limited. Therefore, although an estimate of the consumption of Algal oil (*Chlorella*) in infants from all-proposed food uses has been included for completeness of the data, it is considered to be a substantial over-estimate of the actual expected intake of Algal oil (*Chlorella*) by infants from its use in food.

IV.C. Composition of *Chlorella* Species

C. protothecoides is a species of *Chlorella* that is compositionally similar to other *Chlorella* species such as *C. pyrenoidosa* and *C. vulgaris*. Although the chemical composition of *Chlorella* species is highly variable and is dependent upon the environmental conditions under which the *Chlorella* is grown (Milner, 1948), the compositions of Solazyme's Algal flours (*Chlorella*) are each within the range of results reported for other *Chlorella* species (Robinson and Guzman-Juarez, 1978; Kay, 1991; Brown and Jeffrey, 1992; Tokusoglu and Ünal, 2003; Ravishankar *et al.*, 2006). Additionally, while the majority of the results were obtained from species used in research, the results reported by Kay (1991) were obtained from the analysis of a commercially-available *Chlorella* product (Sun Chlorella). The amino acid composition of Solazyme's high-lipid and high-protein Algal flours (*Chlorella*) is similar to that reported for other *Chlorella* species. The main fatty acids present in Algal high-lipid flour (*Chlorella*), Algal high-protein flour (*Chlorella*), *C. vulgaris*, and *C. pyrenoidosa* are C18:1(as oleic acid), C18:2, and C16:0; however, the quantitative distribution of each fatty acid varies between the products. The genomic sequence of *C. protothecoides* has been compared with that of other commercially-available *Chlorella* products (New Chapter *Chlorella regularis* 390 mg gel caps, Whole Foods Broken Cell Wall *Chlorella* 500 mg pressed tablets, and NutriBiotic CGF 500 mg pressed tablets) (Wolfe *et al.*, 1992; Day *et al.*, 2009). When the four sequences were compared, it was observed that the 23S ribosomal sequence for *C. protothecoides* clustered with that of the commercially-available *Chlorella*. Additionally, there was a high degree of 23S sequence identity between all *Chlorella* analyzed and that of *C. vulgaris*, signifying that *Chlorella* species are genetically similar. The data from the analyses of the chemical composition, amino acid composition, fatty acid composition, and of the genomic sequence of various species of *Chlorella* indicate that the *Chlorella* species are highly similar. The similarity permits the bridging of both toxicological and clinical studies conducted with differing *Chlorella* species to support the safety of *C. protothecoides*.

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IV.D. Fatty Acid Composition of Algal Oil (*Chlorella*) in Comparison to Other Vegetable Oils

The fatty acid composition of Algal oil (*Chlorella*) is qualitatively similar to that of other vegetable oils, such as olive oil, canola oil, and soybean oil, and the lipids provided by Algal oil (*Chlorella*) are constituents of the normal human diet. Table IV.D-1 presents the breakdown of lipids in 4 batches of Algal oil (*Chlorella*), and canola oil, olive oil, and soybean oil.

Fatty Acid Composition	Lot Number						
	Algal Oil (<i>Chlorella</i>)				Olive Oil ^a	Canola Oil ^a	Soybean Oil ^a
	RBD128	RBD138	RDB265	CR227			
C12:0 (AUC%)	0.04	0.03	0.03	NR	0	0	0
C14:0 (AUC%)	1.26	0.77	1.17	0.81	0	0	0
C15:0 (AUC%)	0.06	0.07	0.04	0.04	NR	0	0
C16:0 (AUC%)	11.75	6.97	10.94	10.62	11.29	4.298	10 455
C16:1 (AUC%)	0.54	0.28	0.58	0.95	1.255	0.214	0
C17:0 (AUC%)	0.21	0.14	0.13	0.13	0.022	0	0.034
C18:0 (AUC%)	3.14	2.9	2.37	4.37	1.953	2.087	4.435
C18:1 Oleic (AUC%)	66.47	68.83	70.86	63.31	71.269	61.744	22.55
C18:2 (AUC%)	14.06	17.52	12.22	16.21	9.762	19.005	50.952
C18:3 (AUC%)	1.36	1.03	0.59	2.16	0.761	9.137	6.789
C20:0 (AUC%)	0.36	0.36	0.3	0.45	0.414	0.65	0.361
C20:1 (AUC%)	0.23	0.48	0.22	0.36	0.311	1.317	0.233
C22:0 (AUC%)	0.12	0.08	0.17	0.19	0.129	0.33	0.366
C24:0 (AUC%)	0.06	0.11	NR	0.07	NR	NR	NR
Others (AUC%)	0.18	0.41	0.35	0.31	NR	NR	NR
Total FA (AUC%)	99.98	99.98	99.97	99.98	NR	NR	NR
Total Saturates (AUC%)	17	11.43	15.12	16.68	13.808	7.365	16.65
Total Monounsaturates (AUC%)	67.38	69.59	71.66	64.62	72.961	63.276	22.783
Total Polyunsaturates (AUC%)	15.45	18.55	12.86	18.37	10.523	28.142	57.740
Total Omega 3 (AUC%)	1.36	1.03	0.59	2.16	0	0	0
Total Omega 6 (AUC%)	14.09	17.52	12.22	16.21	0	0	0
Total Omega 9 (AUC%)	66.7	69.31	71.08	63.67	0	0.395	0

NR =not reported

^a Data from USDA's Nutrient Database (USDA, 2009b)

IV.E. Metabolic Fate and Kinetics

Although the absorption, distribution, metabolism, and elimination of Solazyme's Algal oil (*Chlorella*) have not been studied specifically, it is expected that the *Chlorella*-derived lipids will be digested, absorbed, and metabolized through normal physiological processes (PDRNS, 2001), as the fatty acid composition of Algal oil (*Chlorella*) is qualitatively similar to that of other vegetable oils, such as olive oil, canola oil, and soybean oil, and the lipids provided by Algal oil (*Chlorella*) are constituents of the normal human diet.

IV.F. Toxicity Studies

(i) Acute Toxicity Studies

No acute studies for *C. protothecoides* or other *Chlorella* species were identified in the published literature.

(ii) Subchronic Toxicity Studies

Solazyme's high-lipid flour from *C. protothecoides* containing 48% lipid was well-tolerated in a 28-day toxicity study (Day *et al.*, 2009). There were no signs of toxicity and no effect on body weight gains. Although sporadic statistically significant alterations in food consumption, food efficiency ratios, hematological and biochemical parameters, urinalyses, and mean and relative organ weights were noted among males and females, these changes were deemed to be toxicologically irrelevant due to the lack of a dose-response relationship, the fact that they occurred in only one sex, and the lack of supporting gross or microscopic alterations. There were no adverse changes in hematology, coagulation, clinical chemistry, or urinalysis parameters in male or female rats treated with the *Chlorella* high-lipid flour, and there were no effects of treatment on organ weights or on the results of the histopathological analysis. Therefore, under the conditions of the study, the no-observed-adverse-effect level (NOAEL) was the highest concentration tested, 10% algal biomass in the diet, equating to 7,557 and 8,068 mg/kg body weight/day for males and females, respectively. This is equivalent to 3,627 and 3,873 mg/kg body weight/day of Algal oil (*Chlorella*) assuming 48% composition of the algal biomass as the oil.

Several studies evaluating the nutritional value and/or the safety of the dietary administration of *Chlorella* species and powders, including *C. pyrenoidosa* and *C. vulgaris*, in mice, rats, and piglets have been reported. These studies would likely have been conducted on *C. pyrenoidosa* and *C. vulgaris* material containing varying levels of lipid since it is well documented that the protein and lipid contents of *Chlorella* can vary considerably on a dry weight basis based upon differing growing conditions. Although the protein and lipid content can change considerably, comparative studies between species have shown a qualitative similarity in both the amino acid and fatty acid profiles. Based upon the understanding that the *C. pyrenoidosa* and *C. vulgaris* used in the studies would have contained lipids of similar composition to that of

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C. protothecoides, these studies can be used to corroborate the safety of the Algal oil (*Chlorella*).

The studies conducted on *C. pyrenoidosa* and *C. vulgaris* containing 8 to 12.8% lipids include a 45- and 110-day growth study conducted in rats in which gross and histopathological examinations were performed, a 10-week study investigating the effect of *C. vulgaris* on cadmium metabolism that also included 3 non-cadmium treated groups, a 9-week study to examine the effects of *C. vulgaris* on lipid metabolism in rats, a 34-day metabolism study conducted in rats, and 15- and 26-day growth studies conducted in piglets in which hematology and histology were examined (Lubitz, 1963; Khalawan *et al.*, 1980; Yap *et al.*, 1982; Lee *et al.*, 2008; Shim *et al.*, 2009). Although these studies were not conducted consistent with currently accepted toxicology guidelines, the results demonstrate that *Chlorella* species containing lipid provided in the diet support normal growth, are generally well-tolerated, and do not produce any evidence of overt toxicity. Sporadic histological abnormalities were observed in the pancreas and salivary glands of male CD rats provided *Chlorella* 71105 (lipid content not reported) in the diet at a concentration of 21% (~21 to 23 g/kg body weight/day²) or 20.5% (~20.5 g/kg body weight/day¹) with 0.2% methionine for periods of 110 or 45 days; however, not all of the rats were examined histologically, the observed abnormalities were not present in all rats examined, there was no evidence of a dose-response, and the authors noted that "the abnormalities discovered may be artifacts" (Lubitz, 1963). Liver abnormalities (yellow or fatty liver) were reported in 2 male CD rats that received approximately 139 g *Chlorella* 71105/kg body weight/day³ for a period of 37 days; however, this level of *Chlorella* in the diet also had a possible growth-retarding effect and the authors concluded that "the liver abnormality (a yellow or fatty liver) could be a secondary effect of the growth retardation" (Lubitz, 1963). Male Wistar rats that were provided *C. vulgaris* containing 12.8% lipids in the diet at a level of 5 or 10% [equivalent to approximately 5,000 and 10,000 mg/kg body weight/day, respectively (U.S. FDA, 1993)] for 9 weeks had a decreased liver weight relative to body weight compared to the control group (Lee *et al.*, 2008). Although histopathological examinations were not performed, the decrease in relative liver weight was not accompanied by any significant changes in serum aspartate aminotransferase (AST) or alanine transferase (ALT) activities or total protein or bilirubin concentrations. There were no significant differences in liver or kidney weights, serum AST, ALT, or creatinine, urinary creatinine, or creatinine clearance between male CD rats administered *C. vulgaris* containing 12.8% lipids at a concentration of 3 or 5% [equivalent to approximately 3,000 and 5,000 mg/kg body weight/day, respectively (U.S. FDA, 1993)] for 10 weeks compared to control rats (Shim *et al.*, 2009). No signs of clinical toxicity, and no significant differences in hematological or histological parameters or liver weights were observed following the provision of *Chlorella* sp. (lipid content not reported) in the diet of

² Dose calculated using conversion data from U.S. FDA (1993)

³ Based on the assumption of a body weight of 0.1 kg for young rats (U.S. FDA, 1993) and reported food consumption (Lubitz, 1963).

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Yorkshire piglets at a level of 13.81% from Days 4 to 15 or Days 8 to 26 of age (equivalent to approximately 600⁴ and 500⁵ mg/kg body weight/day, respectively) compared to controls (Yap *et al.*, 1982). Similarly, no signs of clinical toxicity, no behavioral changes, and no toxicologically relevant adverse effects upon post-mortem examination were observed in female Harvard rats following the consumption of approximately 9 g *C. pyrenoidosa* (containing 8% lipids)/kg body weight/day⁶ in the diet for 34 days compared to controls (Khalawan *et al.*, 1980). No effects on final body weights, body weight gains, food intake, or food efficiency ratios were observed in rats administered diets supplemented with *Chlorella* species (containing 8 to 14.4% lipids) at concentrations between 5 and 92% [approximately 7,200 to up to 23,157 mg/kg body weight/day (U.S. FDA, 1993)] for durations between 1 and 30 weeks (Lubitz, 1963; Wang *et al.*, 1979, 1980; Khalawan *et al.*, 1980; Saleh *et al.*, 1985; Sano *et al.*, 1988; Herrero *et al.*, 1993; Shibata *et al.*, 2001; Cherng and Shih, 2005). Furthermore, no biologically significant adverse effects on growth or food intake were reported following the provision of diets containing 1% *C. vulgaris* (approximately 1,560 mg/kg body weight/day; lipid content not reported) to mice for 10 weeks, diets containing 7.2% *C. pyrenoidosa* (approximately 8,640 mg/kg body weight/day; containing 13% lipids) to hamsters for 8 weeks, or diets containing 13.81% *Chlorella* sp. (lipid content not reported) to piglets for 11 or 18 days (approximately 492 and 614 mg/kg body weight/day, respectively) (Yap *et al.*, 1982; Chovančíková and Šimek, 2001; Cherng and Shih, 2005). Although not consistently measured in the above studies, no toxicologically significant adverse effects were noted following hematological or biochemical analyses.

The results of an unpublished 2-week toxicity study further support the safety of Solazyme's Algal oil (*Chlorella*) (Krishnaswamy, 2000). No toxicologically significant differences in body weights, food intake, behavior, neurological signs, serum AST or ALT, urinalysis parameters, or gross necropsy or histopathological examinations were reported following the administration of 125, 250, 500, 1,000, or 2,000 mg *C. vulgaris* E25 (containing 10% lipids)/kg body weight/day by gavage to Fischer 344 rats in an escalating dose-pattern compared to controls. Under the conditions of the study, it can be determined that the NOAEL was 2,000 mg/kg body weight/day, the highest dose tested.

A summary of results obtained following short- and long-term oral administration of *Chlorella* to animals is provided in Table IV.F-1.

⁴ Based on the assumption of a food intake of 0.20 kg for individually housed weanling pigs between 0 and 13 days (Varley *et al.*, 2001) and reported body weight (Yap *et al.*, 1982).

⁵ Based on the assumption of a food intake of 0.52 kg for individually housed weanling pigs between 0 and 34 days (Varley *et al.*, 2001) and reported body weight (Yap *et al.*, 1982).

⁶ Based on the assumption of a body weight of 0.1 kg for young rats (U.S. FDA, 1993) and reported food consumption (Khalawan *et al.*, 1980).

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Table IV.F-1 Summary of Subchronic Toxicity Studies on <i>Chlorella</i> sp.						
Species (Strain, Sex, Number of Animals per Group)	Study Duration	Test Article Used and Route of Administration	Dose (mg/kg bw/d)	Parameters Measured Related to Safety	Significant Results Related to Safety ^{a,b}	Reference
<i>C. protothecoides</i>						
Rat (Hsd:Sprague-Dawley SD; 10/sex/group)	28 days	<i>C. protothecoides</i> (containing 48% lipids) [provided in feed at a concentration of 0, 2.5, 5.0, or 10%]	0, 1,794, 3,667, or 7,557 (M) 0, 1,867, 3,918, or 8,068 (F)	<ul style="list-style-type: none"> • Body weight • Food intake • Viability • Signs of clinical toxicity • Behavioral changes • Hematology • Clinical chemistry • Urinalysis • Gross pathology • Macroscopic examination • Histology 	<p>↓ MCHC on Day 29 [7,557, M] ↓ absolute basophil concentration on Day 29 [1,794, M] ↑ absolute large unstained cell concentration on Day 15 [3,918, F] Sporadic ↑ in creatinine, TG, and BUN levels on Day 15 or 29 [≥3,667, M] and serum TC on Day 15 [8,068, F] ↑ serum ALP on Days 15 and 29 [7,557, M] ↓ urine volume, ↑ specific gravity [3,918, F] Blood observed in the urine of 1 mid- and 2 high-dose males. ↑ mean absolute adrenal, adrenal-to-body, adrenal-to-brain weights [3,667, M] Slight liver necrosis in 2 animals [7,557, M] Hepatodiaphragmatic herniation in 1 control rat (M). Minimal to slight mononuclear cell infiltrates in the liver of 5 animals [7,557, M] Minimal mixed cell infiltrate in myocardium of 3 rats [7,557, M] NOAEL [7,557, M; 8,068, F]</p>	Day <i>et al.</i> , 2009

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Table IV.F-1 Summary of Subchronic Toxicity Studies on <i>Chlorella</i> sp.						
Species (Strain, Sex, Number of Animals per Group)	Study Duration	Test Article Used and Route of Administration	Dose (mg/kg bw/d)	Parameters Measured Related to Safety	Significant Results Related to Safety ^{a,b}	Reference
Hamster (Syrian golden; sex not specified, 15/group)	28 days	<i>C. protothecoides</i> (lipid content NR) [provided in high-fat feed at a concentration of 0, 2.5, or 5.0%]	0, 3,000 or 6,000 ^c	<ul style="list-style-type: none"> • Body weight • Food intake • Body composition • Plasma glucose, insulin, TG, TC, HDL-c, protein, albumin 	↓ plasma glucose levels [≥3,000]	Harding and Jones, 2008 [unpublished]
<i>Chlorella</i> 71105						
Rat (CD; 2M)	37 days	<i>Chlorella</i> 71105 (lipid content NR) [provided in feed at a concentration of 92%]	139,243 ^d	<ul style="list-style-type: none"> • Growth • Gross pathology • Histology (eye, heart, lung, liver, brain, kidney, parotid, salivary gland, pancreas, thyroid, and pancreas) 	Normal appearance; a possible growth-retarding effect reported in 1 out of 2 rats. Weight loss reported during the first 3 days of the study but the rats later resumed feeding and gained weight. Histological abnormalities in the pancreas and salivary glands of one rat. Liver abnormalities (yellow/fatty liver) were reported in both rats.	Lubitz, 1963
Rat (CD; 2M)	110 days	<i>Chlorella</i> 71105 (lipid content NR) [provided in feed at a concentration of 21%]	23, 157 ^d		Abnormal cells in pancreas of 1 rat. Abnormal cells in salivary glands of 1 rat.	

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Table IV.F-1 Summary of Subchronic Toxicity Studies on <i>Chlorella</i> sp.						
Species (Strain, Sex, Number of Animals per Group)	Study Duration	Test Article Used and Route of Administration	Dose (mg/kg bw/d)	Parameters Measured Related to Safety	Significant Results Related to Safety ^{a,b}	Reference
Rat (CD; 10M/group)	45 days	T1 <i>Chlorella</i> 71105 (lipid content NR) [provided in feed at a concentration of 21%]	T1: 21,000 ^c		T1: 1 out of the 10 rats developed alopecia after 12 days; condition resolved and all hair grew back 24 days later.	
		T2: <i>Chlorella</i> 71105 (lipid content NR) + L-methionine [provided in feed at a concentration of 20.5% and 0.2%, respectively]	T2: 20,500 ^c		T2: No gross adverse effects reported. Abnormal cells in 1 of the 2 salivary glands examined. 2 out of the 10 rats developed alopecia after 24 days.	
<i>Chlorella</i> sp. (strains not specified)						
Mice (dd; 6 to 9M/group)	7 days	<i>Chlorella</i> sp. (containing 1.51% lipids) [provided in a 2% cholesterol-enriched feed at a concentration of 0 or 10%]	0 or 16,526	<ul style="list-style-type: none"> • Body weight • Food intake • Relative liver weight • Liver lipids • Serum cholesterol 	↓ liver total lipids, TC, and TG levels.	Okuda <i>et al.</i> , 1975
Rat (Wistar; 15 M/group)	10 days	<i>Chlorella</i> sp. (lipid content NR) extract <i>via</i> gavage [following common bile duct ligation and division]	0 or 50	<ul style="list-style-type: none"> • Serum bilirubin, AST, and ALT • Histopathology of terminal ileum 	↑ villous height of terminal ileum	Bedirli <i>et al.</i> , 2009

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Table IV.F-1 Summary of Subchronic Toxicity Studies on <i>Chlorella</i> sp.						
Species (Strain, Sex, Number of Animals per Group)	Study Duration	Test Article Used and Route of Administration	Dose (mg/kg bw/d)	Parameters Measured Related to Safety	Significant Results Related to Safety ^{a,b}	Reference
Rat (Sprague-Dawley; 3 to 7F/group)	Exp 1: 10 weeks	<i>Chlorella</i> sp. (containing 13.76% lipids) [provided in feed at a concentration of 0 or 5%]	0 or 5,000 ^c	<ul style="list-style-type: none"> • Body weight • Serum protein, ALP, ALT, AST • Liver and serum lipids 	Exp 1: ↑ body weight gain. ↓ ALP, ↑ serum TG, ↓ liver TG and TC	Wang <i>et al.</i> , 1979, 1980
	Exp 2: 8 weeks	<i>Chlorella</i> sp. (containing 13.76% lipids) [provided in rice bran basal diet at a concentration of 0 or 5%]	0 or 5,000 ^c		Exp 2: ↑ body weight gain. ↓ liver total lipids, TG.	
	Exp 3: 6 weeks	T1: Basal diet T2: <i>Chlorella</i> sp. (containing 13.76% lipids) [provided in feed at a concentration of 5%] T3: Ethionine [provided in feed at a concentration of 0.25%] T4: <i>Chlorella</i> sp. (containing 13.76% lipids) + ethionine [provided in feed at a concentration of 5% and 0.25%, respectively]	0 or 5,000 ^c	Body weight	T2. ↑ body weight gain (significance not reported)	

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Table IV.F-1 Summary of Subchronic Toxicity Studies on <i>Chlorella</i> sp.						
Species (Strain, Sex, Number of Animals per Group)	Study Duration	Test Article Used and Route of Administration	Dose (mg/kg bw/d)	Parameters Measured Related to Safety	Significant Results Related to Safety ^{a,b}	Reference
Piglet [Yorkshire, M, F; 3 (<i>Chlorella</i>) 4 (control)]	11 days (Days 4 to 15 of age)	<i>Chlorella</i> sp. (lipid content NR) [provided in feed at a concentration of 0 or 13.81%]	0 or 614 ^e	<ul style="list-style-type: none"> • Body weight • Signs of clinical toxicity • Hematology (hemoglobin, serum protein, albumin, urea, and uric acid) 	NSD between groups for any parameter measured and no signs of toxicity.	Yap <i>et al.</i> , 1982
Piglet [Yorkshire; M, F; 4/group]	18 days (Days 8 to 26 of age)	<i>Chlorella</i> sp. (lipid content NR) [provided in feed at a concentration of 0 or 13.81%]	0 or 492 ^f	<ul style="list-style-type: none"> • Histology (stomach, duodenum, ileum, spleen, pancreas, liver, adrenal gland, kidney, colon, cecum, right femur) • Liver weight 	NSD between groups for any parameter measured and no signs of toxicity.	
<i>C. regularis</i>						
Rat (Wistar; 6 M/group)	14 days	<i>C. regularis</i> (containing 8.9% lipids) [provided in feed at a concentration of 0 or 12.7%]	0 or 12,700 ^c	<ul style="list-style-type: none"> • Body weight • Food intake • Liver weight • Serum and liver lipids 	↓serum TC and liver cholesterol content.	Shibata <i>et al.</i> , 2001
<i>C. vulgaris</i>						
Mouse (CDF1; 7 to 10 F/group)	57 days (35 days before and 22 days after Meth A tumor inoculation)	<i>C. vulgaris</i> (lipid content NR) [provided in feed at a concentration of 0, 3, or 10%; before and after Meth A tumor inoculation]	0, 4,500, or 15,000	<ul style="list-style-type: none"> • Body weight • Tumor growth 	No adverse effects or signs of wasting syndrome reported.	Tanaka <i>et al.</i> , 1990

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Table IV.F-1 Summary of Subchronic Toxicity Studies on <i>Chlorella</i> sp.						
Species (Strain, Sex, Number of Animals per Group)	Study Duration	Test Article Used and Route of Administration	Dose (mg/kg bw/d)	Parameters Measured Related to Safety	Significant Results Related to Safety ^{a,b}	Reference
Mouse (CD1; 10 M/group)	10 weeks	<i>C. vulgaris</i> (lipid content NR) [provided in standard feed or high-fat feed at a concentration of 0 or 1%]	0 or 1,560 (standard) 0 or 1,030 (high-fat)	<ul style="list-style-type: none"> • Body weight • Food intake • Liver weight • Serum and liver lipids • Serum ALT 	NSD in parameters measured in mice consuming standard feed. ↓ serum TG and TC/HDL-c ratio, liver TG and TC in mice consuming high-fat feed.	Chovančíková and Šimek, 2001
Rat (Wistar; 12 M/group)	1 week	<i>C. vulgaris</i> (lipid content NR) [provided in a cholesterol-enriched feed at a concentration of 0 or 5%]	0 or 7,500	<ul style="list-style-type: none"> • Liver weight • Serum lipids 	↓ serum TC and PL levels.	Sano <i>et al.</i> , 1988
Rat (F344; 5/sex/group)	2 weeks	<i>C. vulgaris</i> E25 (containing 10%lipids) in deionized water <i>via</i> gavage	Week 1: 0, 125, 250, 500, 1,000, or 2,000 [Doses were doubled during the second week to 0, 250, 500, 1,000, 2,000, and 4,000, respectively]	<ul style="list-style-type: none"> • Body weight • Food intake • Behavioral changes • Signs of clinical toxicity • Neurological examination • Clinical chemistry (serum ALT, AST) • Urinalysis • Organ weights • Gross necropsy • Histology 	Sporadic, non-dose related histopathological changes: <ul style="list-style-type: none"> • Focal round cell collection in myocardium [0, 2,000, M] • Lymphoidal hyperplasia in intestines [0, M, 2,000, F] • Focal areas of liver necrosis [0, F; 125, M; 1,000, M] • Focal round cell collection in liver [125, F; 250, M; 1,000, M] • Round cell collection in glandular stomach [500, F; 1,000 M, F] • Peribronchial round cell collection in lungs [125, M] 	Krishnaswamy, 2000

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Table IV.F-1 Summary of Subchronic Toxicity Studies on <i>Chlorella</i> sp.						
Species (Strain, Sex, Number of Animals per Group)	Study Duration	Test Article Used and Route of Administration	Dose (mg/kg bw/d)	Parameters Measured Related to Safety	Significant Results Related to Safety ^{a,b}	Reference
					<ul style="list-style-type: none"> Varying grades of chronic interstitial pneumonitis in lungs [≥0] 	
Rat (Sprague-Dawley; 6 M/group)	17 days	<i>C. vulgaris</i> (lipid content NR) [provided in feed at a concentration of 0 or 19.6%]	0 or 19,600 ^c	<ul style="list-style-type: none"> Body weight Food intake Plasma uric acid 	Food consumption and body weight data not reported. ↑ plasma uric acid levels.	Saleh <i>et al.</i> , 1985
Rat (Slc:Wistar/ST, 10 M/group)	9 weeks	<i>C. vulgaris</i> (containing 12.8% lipids) [provided in normal or high-fat diet at a concentration of 0, 5, or 10%]	0, 5,000, or 10,000 ^c	<ul style="list-style-type: none"> Body weight Food intake Organ weights (liver, kidney, spleen) Tissue weights (epididymal, perirenal, brown fat pad) Clinical chemistry (AST, ALT, total protein, albumin) Serum and liver lipids Fecal lipid excretion 	<u>Normal diet</u> ↑ body weight gain [≥5,000] ↓ relative liver weight [≥5,000] ↑ fecal wet weight [10,000] ↑ fecal dry weight, and total lipid, TAG, and TC excretion [≥5,000] <u>High-fat diet</u> ↓ relative liver weight [≥5,000] ↓ relative brown fat pad weight [10,000] ↓ serum total lipids [≥5,000] ↓ serum TC and TAG, liver total lipids and TC [10,000] ↓ liver TAG [≥5,000] ↑ fecal dry weight [10,000] ↑ fecal total lipid, TAG, and TC excretion [≥5,000]	Lee <i>et al.</i> , 2008

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Table IV.F-1 Summary of Subchronic Toxicity Studies on <i>Chlorella</i> sp.						
Species (Strain, Sex, Number of Animals per Group)	Study Duration	Test Article Used and Route of Administration	Dose (mg/kg bw/d)	Parameters Measured Related to Safety	Significant Results Related to Safety^{a,b}	Reference
Rat [CD(SD)IGS; 10 M/group]	10 weeks	<i>C. vulgaris</i> (containing 12.8% lipids) [provided in diet at a concentration of 0, 3, or 5%]	0, 3,000, or 5,000 ^c	<ul style="list-style-type: none"> ● Body weight ● Food intake ● Organ weight (liver, kidney) ● Femur weight ● Serum AST, ALT, creatinine ● Urinary creatinine excretion ● Creatinine clearance 	NSD between groups in any of the parameters measured.	Shim <i>et al.</i> , 2009
Rabbit (Japanese white; 8 M/group)	10 weeks	<i>C. vulgaris</i> (lipid content NR) [provided in a cholesterol enriched diet at a concentration of 0 or 1%]	0 or 363	<ul style="list-style-type: none"> ● Body weight ● Organ weights ● Serum lipids ● Aortic lesions 	↓ serum TC, β-lipoprotein levels, and atherosclerotic development.	Sano and Tanaka, 1987
<i>C. pyrenoidosa</i>						
Chick (Hy-line; 10 M/group)	3 weeks	<i>C. pyrenoidosa</i> (containing 11.93% lipids) [provided in feed at a concentration of 0 or 30% ^g]	NR	<ul style="list-style-type: none"> ● Growth 	↓ body weight gain and protein efficiency ratios.	Leveille <i>et al.</i> , 1962
Rat (Holtzman; 10 M/group)	3 weeks	<i>C. pyrenoidosa</i> (containing 11.93% lipids) [provided in feed at a concentration of 0 or 25% ^h]	0 or 25,000 ^c	<ul style="list-style-type: none"> ● Growth 	↓ body weight gain and protein efficiency ratios.	

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Table IV.F-1 Summary of Subchronic Toxicity Studies on <i>Chlorella</i> sp.						
Species (Strain, Sex, Number of Animals per Group)	Study Duration	Test Article Used and Route of Administration	Dose (mg/kg bw/d)	Parameters Measured Related to Safety	Significant Results Related to Safety ^{a,b}	Reference
Rat (Harvard; 3 to 4 F/group)	34 days	<i>C. pyrenoidosa</i> (lipid content NR) [provided in feed at a concentration of 0 or 7%]	0 or 9,249 ^f	<ul style="list-style-type: none"> • Body weight • Signs of clinical toxicity • Behavioral changes • Post-mortem examination (not further defined) 	Larger cecum and smaller fat deposits in the abdominal viscera.	Khalawan <i>et al.</i> , 1980
Rat (F344/DuCRj; 15 M/group)	8 weeks	<i>C. pyrenoidosa</i> (containing 11.2% lipids) [provided in feed at a concentration of 0 or 10%, following a single i.p. injection of DEN]	0 or 6,960 200 (DEN)	<ul style="list-style-type: none"> • Body weight • Food intake • Relative liver weight • GST-P positive foci in the liver 	NSD in the parameters measured.	Takekoshi <i>et al.</i> , 2005
Rat (Wistar; 8 M/group)	2, 4, or 8 weeks	<i>C. pyrenoidosa</i> (containing 13% lipids) [provided in a cholesterol-enriched feed at a concentration of 0, 0.9, 1.8, or 7.2%]	0, 900, 1,800, or 7,200 ^c	<ul style="list-style-type: none"> • Body weight • Food intake • Serum lipids 	<p>↓ serum TG and TC following 2, 4, or 8 weeks [≥900] except NSD in TG at 2 weeks and TC at 4 weeks in [1,800] group</p> <p>↓ serum LDL-c at 2 weeks [900, 7,200], at 4 weeks [7,200], and at 8 weeks [≥900]</p> <p>↑ HDL-c at 4 weeks [7,200]</p> <p>↓ serum TC:HDL-c ratio at 2, 4, or 8 weeks [≥900]</p>	Cherng and Shih, 2005

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Table IV.F-1 Summary of Subchronic Toxicity Studies on <i>Chlorella</i> sp.						
Species (Strain, Sex, Number of Animals per Group)	Study Duration	Test Article Used and Route of Administration	Dose (mg/kg bw/d)	Parameters Measured Related to Safety	Significant Results Related to Safety ^{a,b}	Reference
Hamster (Syrian; 8 M/group)	2, 4, or 8 weeks	<i>C. pyrenoidosa</i> (containing 13% lipids) [provided in a cholesterol-enriched feed at a concentration of 0, 0.9, 1.8, or 7.2%]	0, 1,080, 2,160, or 8,640 ^c	<ul style="list-style-type: none"> • Body weight • Food intake • Serum lipids 	↓ serum TG, TC, and LDL-c at 2, 4, and 8 weeks [≥1,080] except NSD in [2,160] group at 2 weeks ↑ HDL-c at 2, 4, and 8 weeks [≥1,080] ↓ serum TC:HDL-c ratio at 2, 4, and 8 weeks [≥1,080]	
<i>C. stigmatophora</i>						
Rat (Wistar; 10 F/group)	4 weeks	<i>C. stigmatophora</i> (lipid content NR) [provided in feed at a concentration of 0 or 12%]	0 or 17,094	<ul style="list-style-type: none"> • Body weight • Food intake • Hematology • Clinical chemistry • Organ weights (liver, kidneys, heart, lungs, brain, spleen, adrenal gland, thymus) 	NSD in body weight gain, food intake, or hematological parameters. ↓ relative liver and spleen weight ↓ plasma phosphorus, cholesterol and triglyceride levels	Herrero <i>et al.</i> , 1993

↓ = decrease; ↑ = increase; ALP = alkaline phosphatase; ALT = alanine transaminase; AST = aspartate aminotransferase; BUN = blood urea nitrogen; DEN = diethylnitrosamine; F = female; GST-P = glutathione-S-transferase P; HDL-c = high density lipoprotein cholesterol; i.p. = intraperitoneal; LDL-c = low density lipoprotein cholesterol; M = male; MCHC = mean corpuscular hemoglobin concentration; NOAEL = no-observed-adverse-effect level; NR = not reported; NSD = no significant difference; PL = phospholipid; T = treatment; TC = total cholesterol; TG = triglycerides

^a All results are statistically significant and compared to the control group unless otherwise noted.

^b Numbers in [] correspond to the dose(s) at which the reported effects were observed

^c Dose calculated using conversion data from U.S. FDA (1993)

^d Based on the assumption of a body weight of 0.1 kg for young rats (U.S. FDA, 1993) and reported food consumption (Lubitz, 1963).

^e Based on the assumption of a food intake of 0.20 kg for individually housed weanling pigs between 0 and 13 days (Varley and Wiseman, 2001) and reported body weight (Yap *et al.*, 1982).

^f Based on the assumption of a food intake of 0.52 kg for individually housed weanling pigs between 0 and 34 days (Varley and Wiseman, 2001) and reported body weight (Yap *et al.*, 1982).

^g Diets were supplemented with *C. pyrenoidosa* to provide a dietary protein level of 15.31%. The crude protein content of *C. pyrenoidosa* used was 59.96%. (Leveille *et al.*, 1962).

^h Diets were supplemented with *C. pyrenoidosa* to provide a dietary protein level of 18%. The crude protein content of *C. pyrenoidosa* used was 59.96% (Leveille *et al.*, 1962).

ⁱ Based on the assumption of a body weight of 0.1 kg for young rats (U.S. FDA, 1993) and reported food consumption (Khalawan *et al.*, 1980).

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(iii) Reproductive and Teratogenic Toxicity Studies

The absence of adverse reproductive effects from the consumption of *Chlorella* was demonstrated in a three-generation reproduction study conducted with Fzt:DU mice (Janczyk *et al.*, 2006). The provision of a 1.0% spray-dried *C. vulgaris* (containing 8.1% lipids) supplemented diet to mice over 3 generations did not have any effects on adult body weights at age 42 or 63 days, weight of litters at age 0, 10, or 21 days, mean weight of litter mates per litter at age 0, 10, or 21 days, weight of fetuses on Day 16 or 18 of gestation, number of live mouse pups per litter, survival rate of pups from birth to weaning, or number of live, dead, and absorbed fetuses, or corpora lutea in any generation compared to control mice.

The activity of fetal and neonatal hepatic drug metabolizing enzymes and markers of lipid peroxidation were assessed following the administration of *C. vulgaris* (lipid content not reported) by gavage to pregnant and lactating Swiss albino mice for the first 14 days of the gestation and lactation at levels of 0, 100, 300, or 500 mg/kg body weight/day was period (Singh *et al.*, 1998). The livers were excised and assayed for glutathione S-transferase (GST), cytochrome b5, and cytochrome P450 activity and malondialdehyde (MDA) and sulfhydryl (SH) levels. Significantly increased levels of SH and GST were observed in fetal and neonatal livers from doses providing 300 or 500 mg *C. vulgaris*/kg body weight, and significantly decreased hepatic cytochrome b5, cytochrome P450, and MDA levels also were noted in the developing fetuses and neonatals whose mothers were administered 500 mg/kg body weight/day. The dose of 100 mg/kg body weight/day by gavage had no effect on hepatic SH, GST, cytochrome b5, cytochrome P450, or MDA levels. No other treatment-related effects were reported.

Diets supplemented with 7% *C. pyrenoidosa* (lipid content not reported) and 50% *Saccharomyces cerevisiae* provided to Sprague-Dawley rats over 2 generations were readily accepted and the number of offspring born and weaned was similar between the treatment group and the control group (not statistically analyzed) (Khalawan *et al.*, 1980). Due to insufficient quantities of diet for the growing rat colony, the rats were replaced with mice in order to continue the study. Albino mice were provided the same experimental diet as the rats or a commercial diet over 4 generations and the average food consumption was noted to be similar between the 2 dietary groups, with the experimental animals consuming approximately 0.35 g *C. pyrenoidosa*/day (14 g/kg body weight/day, based on an average body weight of 25 g). There were no signs of toxicity among the mice, and growth was unaffected by the diet treatments. Additionally, the number of offspring born and weaned appeared to be similar between the dietary groups (not statistically analyzed). The addition of *C. vulgaris* (lipid content not reported) in the diet (dose not reported) of breeding sows and piglets (strain not reported) for an unspecified duration had no significant effects on feed intake, reproductive performance, or productive performance (Köhler *et al.*, 2008).

(iv) Mutagenicity and Genotoxicity Studies

No mutagenicity or genotoxicity studies were identified in the literature.

(v) Carcinogenicity Studies

No traditional carcinogenicity studies were identified in the literature; however, the effects of *Chlorella* sp. on tumor growth have been investigated in mice and rats. *C. vulgaris* dried powder (lipid content not reported) or its acetone extract administered in the diet for up to 57 days at levels of up to 15 g/kg body weight/day did not promote the growth of subcutaneously inoculated 3-methylcholanthrene-induced tumor cells in CDF1 mice (Tanaka *et al.*, 1990). Additionally, the authors reported that dietary administration of *C. vulgaris* dried powder resulted in no serious side effects including decreases in body weights or other wasting syndromes. Dietary *C. pyrenoidosa* (lipid content not reported) at a concentration of 10% (equivalent to approximately 6,960 mg/kg body weight/day) administered for 59 days was observed to have an inhibitory effect on hepatocarcinogenesis in male F344/DuCrj rats initiated and/or promoted with diethylnitrosamine and 2-amino-3,8-dimethylimidazo[4,5-f] quinoxaline, respectively (Takekoshi *et al.*, 2005). Bone marrow colony formation was significantly increased and survival was prolonged in male BALB/c mice inoculated with Erlich ascites tumor following the administration of *C. vulgaris* extract by gavage for 5 days at doses of 50, 100, or 200 mg/kg body weight/day compared to placebo tumor-bearing mice (Justo *et al.*, 2001).

(vi) Human Studies

The consumption of *Chlorella* species containing 11 to 19% lipids by humans was reported to be well-tolerated in a number of studies in which the beneficial effects of the algae on the immune system, hypertension, fibromyalgia syndrome, ulcerative colitis, and glioma (primary brain tumors) were investigated, as well as in studies where *Chlorella* replaced dietary high-quality protein sources such as fish, egg, and soy as the principle source of nitrogen consumption (Dam *et al.*, 1965; Lee *et al.*, 1967; Merchant *et al.*, 1990, 2000, 2002; Merchant and Andre, 2001; Halperin *et al.*, 2003). The only adverse effects reported following the consumption of up to 90 g of *Chlorella* species for periods of up to 2 years were feelings of fatigue following the consumption of 200 mg *C. pyrenoidosa* (lipid content not reported) per day for a period of 28 days (Halperin *et al.*, 2003) and symptoms of gastrointestinal upset, nausea, and fever during the first week of treatment with 20 g of *C. pyrenoidosa* (containing approximately 11% lipids) and 150 mL of a liquid *C. pyrenoidosa* extract (Merchant *et al.*, 1990). The symptoms of gastrointestinal upset were noted to generally subside over the rest of 2-year study period. In a 26-day study in which 5 healthy males (aged 18 to 23 years) were provided a mixture of *Chlorella* and *Scenedesmus* (ratio not reported) containing 19% lipids in gingerbread, chocolate cake, chocolate cookies, and milk in increasing amounts from 10 to up to 500 g/day, the authors concluded that the "algae in amounts up to 100 g/man/day can be well-tolerated at least for a short time" (Powell *et al.*, 1961). When provided at levels greater than 100 g/day, the

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volunteers had difficulty digesting the test items and experienced abdominal distention, associated with increased erucation and flatulence. Nausea, mild abdominal cramping pain, headache, malaise, and hard bulky stools were reported by the subjects when the level of algae consumed reached 500 g algae/day. No abnormalities were reported in physical examinations other than those associated with the gastrointestinal tract, and hematology, urinalysis, and liver function tests were all within normal limits. Similarly, no adverse effects or significant differences in hematology, clinical chemistry, or urinalysis parameters were reported to occur in subjects with fibromyalgia syndrome, ulcerative colitis, mild to moderate hypertension, or malignant gliomas following the consumption of up to 20 g *Chlorella* (containing approximately 11% lipids) and 150 mL *Chlorella* extract for periods of up to 2 years (Merchant *et al.*, 1990, 2000, 2002; Merchant and Andre, 2001).

Photosensitive dermatitis is the only significant adverse effect that has been reported following the consumption of *Chlorella* species in humans. This effect was determined to result from the presence of pheophorbide a, a breakdown product of chlorophyll a (Jitsukawa *et al.*, 1984; Jassby, 1988). An investigation by Tokyo Bureau of Metropolitan Health revealed that 23 cases of photosensitive dermatitis that occurred between June 1976 and June 1977 occurred in persons consuming a specific brand of *Chlorella*, "Kenbi *Chlorella*" (Jitsukawa *et al.*, 1984). Only *Chlorella* products produced between April 1976 and April 1977 were reported to cause photosensitive dermatitis, which coincided with a change in the manufacturing process during the drying process (moistening the *Chlorella* powder with water and ethanol followed by drying at 90°C for 30 minutes). As the enzyme responsible for pheophorbide-a production, chlorophyllase, is reported to have a high activity at 80°C but no activity at 100°C, the change in manufacturing was determined to cause the production of pheophorbide-a. In 1981, the Japanese Public Health Ministry recommended that the level of pheophorbide a in algae preparations be restricted to less than 1.2 mg/g (Becker, 1994). The analysis of 2 non-consecutive lots of Solazyme's Algal flour (*Chlorella*) (1 high-protein and 1 high-lipid) demonstrated that the level of pheophorbide-a in Solazyme's Algal flour (*Chlorella*) (not detected to 0.0334 mg/g) is below the limit established by the Japanese Public Health Ministry. The results of the studies conducted in humans with the oral administration of various species of *Chlorella* do not indicate any potential for toxicity or cause for concern resulting from the consumption of Solazyme's Algal oil (*Chlorella*) under the conditions of intended use.

A summary of results obtained following oral administration of *Chlorella* to humans is provided in Table IV.F-2.

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Table IV.F-2 Summary of Human Safety Studies on <i>Chlorella</i> Species							
Study Design	Final Population (age, sex, health status)	Duration of Treatment	Test item	Daily Dose	Parameters Measured Related to Safety	Significant Results Related to Safety ^{a,b}	Reference
<i>C. pyrenoidosa</i>							
NR, NB, NC	6 subjects (18 to 32 yrs; 3 M, 3 F; healthy)	5 days	Various foods in the diet Lipid content of <i>C. pyrenoidosa</i> NR	57.3 g ^c	NE	No adverse events were reported by the authors.	Lee <i>et al.</i> , 1967
NR, NB, NC, CO	5 subjects (24 to 35 yrs; 4 M, 1 F; healthy)	10 days	Ethanol-extracted algae in biscuits and pizza Lipid content of <i>C. pyrenoidosa</i> NR	54.2 and 90.3 g	Adverse events	1 subject withdrew from the study (reason not specified). No reports of nausea, bloated feeling, or bitter taste were attributed to treatment.	Dam <i>et al.</i> , 1965
R, DB, C	124 subjects (50 to 89 yrs; 29 M, 95 F; healthy)	28 days	Aqueous extract in capsules	0 (placebo), 200, or 400 mg A trivalent influenza vaccine was administered on Day 21	Adverse events Immunological parameters (antibody response to influenza vaccine) Liver enzymes (not further specified) Complete blood counts (not further specified)	7 subjects withdrew from the study: <ul style="list-style-type: none"> • 2 from [200] group (1 left the country, 1 due to ill health); • 5 from [400] group [1 due to adverse events (nausea and abdominal discomfort), 1 due to physician's advice, 1 did not want vaccine, 2 due to ill health]. NSD in incidence of fever, rash, headache, body aches, sore joints, abdominal pain, nausea, anorexia, vomiting, and diarrhea between groups. ↑ incidence of fatigue [200] vs. placebo and	Halperin <i>et al.</i> , 2003

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Table IV.F-2 Summary of Human Safety Studies on <i>Chlorella</i> Species							
Study Design	Final Population (age, sex, health status)	Duration of Treatment	Test item	Daily Dose	Parameters Measured Related to Safety	Significant Results Related to Safety ^{a,b}	Reference
						[400]. NSD in overall antibody response to influenza vaccination. ↑ antibody response in subjects 50 to 55 yrs [400] vs. those given placebo. Liver enzyme and blood count results not reported.	
NR, NB, NC	20 subjects (18 to 65 yrs; 1 M, 19 F; fibromyalgia syndrome)	2 months	Tablet (containing 11% lipids) and liquid extract	10 g (tablet) and 100 mL (extract)	Adverse events Serum chemistry (parameters not specified) Hematology (parameters not specified)	2 subjects withdrew from the study: <ul style="list-style-type: none"> • 1 due to nausea following treatment; • 1 did not want to participate in the study. ↑ frequency of diarrhea and abdominal cramping reported; symptoms did not require medical intervention and did not limit activity of subjects. No effect on serum chemistry or hematology parameters.	Merchant <i>et al.</i> , 2000

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Table IV.F-2 Summary of Human Safety Studies on <i>Chlorella</i> Species							
Study Design	Final Population (age, sex, health status)	Duration of Treatment	Test item	Daily Dose	Parameters Measured Related to Safety	Significant Results Related to Safety ^{a,b}	Reference
NR, NB, NC	98 subjects (25 to 56 yrs; sex not specified; ulcerative colitis)	2 months	Tablet (containing 11% lipids) and liquid extract	10 g (tablet) and 100 mL (extract)	Physical examinations Hematology (parameters not specified)	1 subject dropped out of the study (reason not specified). NSD in physical examination results and hematological parameters. No adverse effects on the symptoms of ulcerative colitis reported.	Merchant and Andre, 2001
NR, NB, NC	34 subjects [normal group: 34.3±3.2 yrs, high-risk group: 59.2±1.9 yrs; M; healthy] ^d	12 weeks (with 4 month follow-up)	Tablet (lipid content NR)	7.64 g ^e	Physical examinations FBG Serum lipids (TC, LDL-c, HDL-c)	1 normal subject dropped out due to stomach pains. No subjects reported any complications that could be considered to be harmful side effects during physical examinations. ↓ FBG at 8 weeks vs. baseline levels in high-risk subjects. ↓ FBG at 12 and 16 weeks vs. baseline in normal subjects. ↓ TC, LDL-c, and HDL-c at 4, 8, 12, and 16 weeks vs. baseline levels in high-risk subjects. ↓ TC and HDL-c at 4 and 8 weeks, ↓ LDL-c at 4 weeks, vs. baseline in normal subjects.	Mizoguchi <i>et al.</i> , 2008

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Table IV.F-2 Summary of Human Safety Studies on <i>Chlorella</i> Species							
Study Design	Final Population (age, sex, health status)	Duration of Treatment	Test item	Daily Dose	Parameters Measured Related to Safety	Significant Results Related to Safety ^{a,b}	Reference
NR, SB, C (1-month placebo washout period of antihypertensive medication prior to treatment)	24 subjects (22 to 73 yrs; 11 M, 13 F; mild to moderate hypertension)	32 months	Tablet (containing 11% lipids) and liquid extract	10 g (tablet) and 100 mL (extract)	Adverse events Physical examinations Hematology (parameters not specified) Clinical chemistry (parameters not specified) Urinalysis (parameters not specified) Serum lipids (TC, TG, HDL-c, LDL-c)	1 subject withdrew after 4 weeks as his mean BP was too high. NSD in physical examination results, body weight, ECG findings, serum clinical chemistry, hematology, or urinalysis parameters, NSD in HR, systolic BP, and diastolic BP vs. placebo period. ↓serum TC and LDL-c vs. baseline and placebo periods. ↓ HDL-c vs. placebo period.	Merchant and Andre, 2001; Merchant <i>et al.</i> , 2002
R, DB, CO, C (1-month washout period before crossover)	37 subjects (47.1±9.0 yrs; 36 F, 1 M; fibromyalgia syndrome)	3 months	Tablet (containing 11% lipids) and liquid extract	10 g (tablet) and 100 mL (extract)	Physical examinations Hematology (parameters not specified) Urinalysis (parameters not specified)	NSD in physical examination results, and hematological and urinalysis parameters. Adverse events were not reported by the authors.	Merchant and Andre, 2001
NR, NB, NC	20 subjects (19 to 69 yrs; sex not specified; malignant glioma)	Up to 2 years	Tablet (containing 11% lipids) and liquid extract	20 g (tablet) and 150 mL (extract)	Adverse events Hematology (RBC, WBC, hemoglobin, hematocrit, MCHC, MCV, platelet count) Immunological parameters (circulating concentrations of	No adverse effects attributed to long-term supplementation. Transient adverse effects reported at the beginning of treatment, which resolved within a few days to a week, and included: • 8/21 subjects (38%) experienced nausea	Merchant <i>et al.</i> , 1990

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Table IV.F-2 Summary of Human Safety Studies on <i>Chlorella</i> Species							
Study Design	Final Population (age, sex, health status)	Duration of Treatment	Test item	Daily Dose	Parameters Measured Related to Safety	Significant Results Related to Safety ^{a,b}	Reference
					monocytes, leukocytes, and granulocytes, proportion of lymphocytes bearing specific T-cell and natural killer cell markers)	or slight fever; • 6/21 subjects (29%) reported irregular bowel movements, intestinal cramping, ↑ flatus; • 3/21 subjects (14%) experienced constipation and nausea; 1 subject withdrew from the study due to aversion to the taste of <i>Chlorella</i> , which developed as a result of nausea from radiotherapy. No adverse effects on hematological and immunological parameters measured were reported. Adverse changes in clinical status usually correlated with CT scan or MRI evidence of tumor recurrence and/or progressive growth and were not attributed to <i>Chlorella</i> supplementation.	

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Table IV.F-2 Summary of Human Safety Studies on <i>Chlorella</i> Species							
Study Design	Final Population (age, sex, health status)	Duration of Treatment	Test item	Daily Dose	Parameters Measured Related to Safety	Significant Results Related to Safety ^{a,b}	Reference
<i>C. regularis</i>							
NR	20 subjects (mean age of 53 yrs; 12 F, 8 M; type II hypercholesterolemia)	3 months	Tablet (containing 11% lipids)	3 g	Adverse events Blood pressure Body weight Serum lipids (TC, LDL-c, HDL-c, Apo A-I, Apo-B)	No side effects reported during study period. NSD in body weight, and systolic and diastolic blood pressure. ↓ serum TC and LDL-c at 1 and 3 months vs. baseline ↑ serum Apo A-I at 3 months vs. baseline. ↓ atherogenic index (Apo B/Apo A-I) at 3 months vs. baseline.	Sansawa <i>et al.</i> , 2002
<i>C. vulgaris</i>							
R, DB, C	52 males (20 to 65 yrs; healthy)	6 weeks	Pill (lipid content NR)	6.3 g	NE	Adverse events were not reported by the authors.	Lee <i>et al.</i> , 2010
<i>Chlorella</i> (strain not specified)							
NR	16 subjects (gender NR; hypercholesterolemia)	3 months	Tablet (containing 1.51% lipids)	5 g	Serum cholesterol	↓ serum cholesterol levels. Adverse events were not reported by the authors.	Okuda <i>et al.</i> , 1975
NR	23 subjects (mean age of 53.8 yrs; 11 F, 12 M; hypertensive)	6 months	Tablet (containing 10% lipids)	1.5 g	Adverse events Blood pressure Hematology (RBC, WBC, hemoglobin, hematocrit, platelet count) Clinical chemistry (FBG, uric acid, Na, K, Cl, BUN, ALP, GPT, GOT, total protein, TTT, ZnTT)	NSD in adverse events, arrhythmia symptoms, and myocardial ischemia symptoms. ↓ systolic and diastolic blood pressure vs. baseline. ↓ serum TC and TG at 6 months vs. baseline. ↑ serum HDL-c at 6 months vs. baseline. ↓ serum uric acid and	Inoue <i>et al.</i> , 1995

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Table IV.F-2 Summary of Human Safety Studies on <i>Chlorella</i> Species							
Study Design	Final Population (age, sex, health status)	Duration of Treatment	Test item	Daily Dose	Parameters Measured Related to Safety	Significant Results Related to Safety ^{a,b}	Reference
					Serum lipids (TC, TG, HDL-c)	FBG at 6 months vs. baseline.	
<i>Chlorella</i> and <i>Scenedesmus</i> (strains not specified)							
NR	5 healthy males (18 to 23 yrs)	26 days	<i>Chlorella</i> (containing 19% lipids) and <i>Scenedesmus</i> autoclaved and incorporated into gingerbread, chocolate cake, chocolate cookies, and milk	Increased gradually to 500 g	Tolerability Physical examinations Hematology (parameters not specified) Urinalysis (parameters not specified) Liver function tests (parameters not specified)	Authors concluded that algae was well-tolerated at levels up to 100 g algae/day. Difficulty digesting the test items at levels greater than 100 g algae/day. No abnormalities in physical examinations other than those associated with the gastrointestinal tract. Hematology, urinalysis, and liver function tests were all within normal limits. Adverse events observed included: <ul style="list-style-type: none"> Abdominal distention, associated with increased erucation and flatulence, early in the study. Increased bowel movements with bulky and dry stools at levels greater than 50 g algae/day. These effects became more severe at levels greater than 200 g algae/day.	Powell <i>et al.</i> , 1961; Krauss, 1962

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Table IV.F-2 Summary of Human Safety Studies on <i>Chlorella</i> Species							
Study Design	Final Population (age, sex, health status)	Duration of Treatment	Test item	Daily Dose	Parameters Measured Related to Safety	Significant Results Related to Safety ^{a,b}	Reference
						<ul style="list-style-type: none"> • Nausea, mild abdominal cramping pain, headache, malaise, and hard bulk stools at level of 500 g algae/day. 2 subjects dropped out of the study: <ul style="list-style-type: none"> • 1 due to diffuse lower abdominal cramping pains, increased flatulence, nausea, and persistent vomiting at level of 200 g algae/day. • 1 due to similar effects at a level of 500 g algae/day • All adverse effects disappeared 48 hours after discontinuing algae supplementation. 	

↓ = decrease; ↑ = increase; ALP = alkaline phosphatase; Apo = apolipoprotein; BP = blood pressure; BUN = blood urea nitrogen; C = controlled; Cl = chloride; CO = crossover; CT = computed axial tomography; DB = double-blind; ECG = electrocardiogram; F = female; FBG = fasting blood glucose; GOT = glutamic oxaloacetic transaminase; GPT = glutamic pyruvic transaminase; HDL-c = high-density lipoprotein cholesterol; HR = heart rate; K = potassium; LDL-c = low-density lipoprotein cholesterol; M = male; MCHC = mean corpuscular hemoglobin concentration; MCV = mean cell volume; MRI = magnetic resonance imaging; Na = sodium; NB = non-blinded; NC = non-controlled, NE = not evaluated; NSD = no significant difference; NR = non-randomized; R = randomized; SB = single blind; TC = total cholesterol; TG = triglycerides; TTT = thymol turbidity test; ZnTT = zinc turbidity test

^a All results are statistically significant unless otherwise noted.

^b Numbers in [] correspond to the dose(s) at which the reported effects were observed

^c Diet was supplemented with *C. pyrenoidosa* to provide 6.0 g N/day; the algae used in the study had protein content of 65.4% (N x 6.25) (Lee *et al.*, 1967).

^d Healthy subjects were considered to be at high-risk for life-style related diseases if they had borderline high FBG, low glucose tolerance, high total blood serum cholesterol, and/or high serum TG (Mizoguchi *et al.*, 2008).

^e Subjects were given 40 "Sun Chlorella A" tablets/day; one tablet weighs 200 mg and contains >95.5% dried *Chlorella* powder as the active ingredient (Mizoguchi *et al.*, 2008).

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IV.G. Algal Cyanobacterial Toxin and Pheophorbide Analysis

None of the algal or cyanobacterial toxins that have been identified in the published literature or mentioned in international food regulations [*i.e.*, amnesic shellfish poisoning toxins (Domoic acid), paralytic shellfish poisoning (PSP) toxins (N-sulfocarbamoyl toxins C1-4, B1, B2, decarbamoylgonyautoxins 1-4, gonyautoxins 1-4, decarbamoylsaxitoxin, saxitoxin, and neosaxitoxin), diarrhetic shellfish poisoning toxins (okadaic acid, dinophysistoxins, pectenotoxins, yessotoxins, azaspiracides, and gymnodimines), neurotoxic shellfish poisoning toxins (brevetoxins), and the cyanobacterial toxins (microcystins MC-RR, -LR, -YR, -LA, -LF, -LW, nodularin (NOD), anatoxin-a, cylindrospermopsins (CYN), and β -methylamino- L-alanine (BMAA)] were detected in Solazyme's Algal high-lipid flour (*Chlorella*) (Day *et al.*, 2009). The level of pheophorbide a, a breakdown product of chlorophyll a reported to cause photosensitive dermatitis in humans, in Solazyme's Algal flour (*Chlorella*) (below detection to 0.0334 mg/g) is considerably lower than the limit established by the Japanese Public Health Ministry (1.2 mg/g).

IV.H. Allergy

Limited data regarding the potential allergenicity of *Chlorella* species were identified in the literature (Tiberg *et al.*, 1990a,b, 1995). The results of these studies indicate that proteins of *C. vulgaris*, *C. saccharophila*, or *C. homosphaera* do not have significant allergenic potential, even in atopic individuals. It is important to note that no reports of allergy were identified in the scientific literature following consumption of *Chlorella*. The long history of safe consumption of *Chlorella* products strongly supports the lack of allergenic potential.

IV.I. Summary and Basis for GRAS

Solazyme intends to market Algal oil (*Chlorella*) produced from the dried biomass of *C. protothecoides* S106 as a food ingredient in a variety of traditional food and beverage products. Algal oil (*Chlorella*) is manufactured according to cGMP and all media components and processing aids are suitable for use in food production. All media components and processing aids are removed through extensive purification processes. The source strain, *C. protothecoides* S106, and the master and working cell banks used in the production of Algal oil (*Chlorella*) were characterized by molecular methods, which showed they were genetically identical. Analytical data support that Algal oil (*Chlorella*) is consistently manufactured to suitable food grade specifications, including limits set for residual solvents and heavy metals. Algal oil (*Chlorella*) is stable for up to 148 days under accelerated testing conditions based on the results of an ongoing stability study conducted with Algal high-lipid flour (*Chlorella*). A 12-month shelf-life has been designed for Algal oil (*Chlorella*) and formal accelerated stability studies are ongoing.

Algal oil (*Chlorella*) is intended to be used as an ingredient in baked goods and baking mixes, beverages (alcoholic), beverages and beverage bases, confections and frostings, fats and oils, frozen dairy desserts and mixes, gelatins, puddings, and fillings, grain products and pastas, milk

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products, nut and nut products, processed fruits and fruit juices, snack foods, soft candy, soups and soup mixes, and sweet sauces, toppings, and syrups at use-levels ranging from approximately 1.8 to 26%, with the exception of frying, cooking, and specialty oils at a use-level of 100%. Using data collected in the NHANES 2005-2006 and under the conditions of intended use of Algal oil (*Chlorella*), the total U.S. population mean all-user intake of Algal oil (*Chlorella*) was 20.1 g/person/day on an absolute basis or 0.35 g/kg body weight/day on a body weight basis. The 90th percentile all-user intake of Algal oil (*Chlorella*) from all proposed food-uses by the total population was 39.4 g/person/day, or 0.73 g/kg body weight/day on a body weight basis. Although Algal oil (*Chlorella*) is intended for use in all food categories indicated, it is highly unlikely that Algal oil (*Chlorella*) will be used in all food categories and all food-uses simultaneously due to the numerous other vegetable oils currently available on the market. In a more realistic scenario, Algal oil (*Chlorella*) is expected to achieve levels of use similar to those of olive, canola, sunflower, or peanut oil, which have a daily *per capita* intake ranging from 0.25 (sunflower seed oil) to 11.7 (canola oil) g/person/day. The daily *per capita* intake of canola oil is approximately half the estimated total population mean all-user intake of Algal oil (*Chlorella*) and approximately 3 times lower than the estimated total population 90th percentile all-user intake of Algal oil (*Chlorella*). Therefore, it is expected that the estimated intakes for Algal oil (*Chlorella*) are substantial over-estimates of the anticipated actual consumption and that the actual levels of consumption will be more similar to the levels of olive, peanut, or sunflower oils. Furthermore, based on the limitations of the methodology used to estimate Algal oil (*Chlorella*) consumption, which include the use of short-term surveys, inclusion of numerous infrequently consumed foods, and the assumption that all food products within a food-use contain Algal oil (*Chlorella*) at the maximum specified level of use, it is reasonable to conclude that these intake estimates represent substantial overestimates of the actual Algal oil (*Chlorella*) exposure in the U.S. population that are expected under the proposed uses in food described herein.

The safety of Solazyme's Algal oil (*Chlorella*) under the proposed uses was based on scientific procedures using generally available data. Solazyme's *C. protothecoides* is compositionally similar to other *Chlorella* species used in research and commercially-available (*i.e.*, Sun Chlorella). The amino acid composition of Solazyme's Algal high-lipid and high-protein flour (*Chlorella*) is similar to that reported for other *Chlorella* species. The main fatty acids present in Algal high-lipid flour (*Chlorella*), Algal high-protein flour (*Chlorella*), *C. vulgaris*, and *C. pyrenoidosa* are C18:1 (as oleic acid), C18:2, and C16:0; however, the quantitative distribution of each fatty acid varies between the products. The genomic sequence of *C. protothecoides* has been compared with that of other commercially-available *Chlorella* products (New Chapter *Chlorella regularis* 390 mg gel caps, Whole Foods Broken Cell Wall *Chlorella* 500 mg pressed tablets, and NutriBiotic CGF 500 mg pressed tablets) (Wolfe *et al.*, 1992; Day *et al.*, 2009). When the four sequences were compared, it was observed that the 23S ribosomal sequence for *C. protothecoides* clustered with that of the commercially-available *Chlorella*. Additionally, there was a high degree of 23S sequence identity between all *Chlorella* analyzed and that of *C. vulgaris*, signifying that *Chlorella* species are genetically similar. The data from the analyses

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of the chemical composition, amino acid composition, fatty acid composition, and of the genomic sequence of various species of *Chlorella* indicate that the *Chlorella* species are highly similar. The similarity permits the bridging of both toxicological and clinical studies conducted with differing *Chlorella* species to support the safety of *C. protothecoides*.

Published studies indicate that *Chlorella* species are of low oral toxicity in animals. The NOAEL in a 28-day toxicity study with Solazyme's high-lipid Algal flour (*Chlorella*) was the highest tested concentration, 10% algal biomass in the diet, equating to 7,557 and 8,068 mg/kg body weight/day for males and females, respectively (Day *et al.*, 2009). This is equivalent to 3,627 and 3,873 mg/kg body weight/day of Algal oil (*Chlorella*) assuming 48% composition of the algal biomass as the oil. In several studies which evaluated the nutritional value and/or the safety of *Chlorella* species (containing 8 to 12.8% lipids), including *C. pyrenoidosa* and *C. vulgaris*, in the diet of mice, rats, and piglets, it was demonstrated that *Chlorella* species provided in the diet support normal growth, are generally well-tolerated, and do not produce any evidence of overt toxicity (Lubitz, 1963; Khalawan *et al.*, 1980; Yap *et al.*, 1982; Lee *et al.*, 2008; Shim *et al.*, 2009). The absence of adverse reproductive effects from the consumption of a diet containing 1% spray-dried *Chlorella* (containing 8.1% lipids) was demonstrated in a three-generation reproduction study conducted in Fzt:DU mice (Janczyk *et al.*, 2006). No traditional genotoxicity or 2-year carcinogenicity studies were identified in the literature; however, anti-tumor effects have been demonstrated in mice and rats.

The consumption of *Chlorella* species containing 11 to 19% lipids by humans was reported to be well-tolerated in a number of studies in which the beneficial effects of the algae on the immune system, hypertension, fibromyalgia syndrome, ulcerative colitis, and glioma (primary brain tumors) were investigated, as well as in studies where *Chlorella* replaced dietary high-quality protein sources such as fish, egg, and soy as the principle source of nitrogen consumption (Dam *et al.*, 1965; Lee *et al.*, 1967; Merchant *et al.*, 1990, 2000, 2002; Merchant and Andre, 2001; Halperin *et al.*, 2003). The only adverse effects reported following the consumption of up to 90 g of *Chlorella* species for periods of up to 2 years were feelings of fatigue following the consumption of 200 mg *C. pyrenoidosa* per day for a period of 28 days (Halperin *et al.*, 2003) and symptoms of gastrointestinal upset, nausea, and fever during the first week of treatment with 20 g of *C. pyrenoidosa* and 150 mL of a liquid *C. pyrenoidosa* extract in a 2-year study (Merchant *et al.*, 1990).

None of the algal or cyanobacterial toxins that have been identified in the published literature or mentioned in international food regulations [*i.e.*, amnesic shellfish poisoning toxins (Domoic acid), PSP toxins (N-sulfocarbamoyl toxins C1-4, B1, B2, decarbamoylgonyautoxins 1-4, gonyautoxins 1-4, decarbamoylsaxitoxin, saxitoxin, and neosaxitoxin), diarrhetic shellfish poisoning toxins (okadaic acid, dinophysistoxins, pectenotoxins, yessotoxins, azaspiracides, and gymnodimines), neurotoxic shellfish poisoning toxins (brevetoxins), and the cyanobacterial toxins (microcystins MC-RR, -LR, -YR, -LA, -LF, -LW, NOD, anatoxin-a, CYN, and BMAA)] were

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detected in Solazyme's Algal high-lipid flour (*Chlorella*) (Day *et al.*, 2009). The level of pheophorbide a, a breakdown product of chlorophyll a reported to cause photosensitive dermatitis in humans, in Solazyme's Algal flour (*Chlorella*) (below detection to 0.0334 mg/g) is considerably lower than the limit established by the Japanese Public Health Ministry (1.2 mg/g).

General recognition of safety for the use of Solazyme's Algal oil (*Chlorella*) for use in a food as described in Appendix A at use levels of between 1.8 to 26% and 100% in frying, cooking, and specialty oils, is based on the opinion of an Expert Panel of scientists qualified by scientific training and experience to evaluate the safety of Algal oil (*Chlorella*) for use in food. The Expert Panel independently and collectively critically evaluated the data and information summarized above and concluded that the intended uses as a food ingredient of Algal oil (*Chlorella*), meeting appropriate food-grade specifications described herein and produced consistent with cGMP, are safe and suitable. They further concluded that the intended uses of Algal oil (*Chlorella*), meeting appropriate food-grade specifications presented herein and produced consistent with cGMP, are GRAS based on scientific procedures. It also is Solazyme's opinion that other qualified experts would concur with these conclusions.

V. Conclusion

Based on the data and information summarized above, it can be concluded that Solazyme's Algal oil (*Chlorella*) produced from *C. protothecoides* S106, meeting appropriate food-grade specifications and manufactured in accordance with cGMP, is GRAS for the intended uses in traditional food products as described herein based on scientific procedures.

Therefore, the use of Solazyme's Algal oil (*Chlorella*) in food as described herein is exempt from the requirement of premarket approval (Section 409 of the *Federal Food, Drug and Cosmetic Act*) (U.S. FDA, 2009c).

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Table of CFR Sections Referenced (Title 21—Food and Drugs)		
Part	Section §	Section Title
101—Food Labeling	101.12	Reference amounts customarily consumed per eating occasion
102—Common or usual name for nonstandardized foods	102.22	Protein hydrolysates
168—Sweeteners and table syrups	168.120	Glucose sirup
170—Food additives	170.3	Definitions
	170.30	Eligibility for classification as generally recognized as safe (GRAS)
172—Food additives permitted for direct addition to food for human consumption	172.185	TBHQ
	172.808	Copolymer condensates of ethylene oxide and propylene oxide.
173—Secondary direct food additives permitted in food for human consumption	173.240	Isopropyl alcohol
	173.270	Hexane
	173.340	Defoaming agents
176—Indirect food additives: Paper and paperboard components	176.180	Components of paper and paperboard in contact with dry food
177—Indirect food additives: Polymers	177.2420	Polyester resins, cross-linked
182—Substances generally recognized as safe	182.3149	Ascorbyl palmitate
	182.3890	Tocopherols
	182.6285	Dipotassium phosphate
	182.8159	Biotin
	182.8252	Choline chloride
184—Direct food substances affirmed as generally recognized as safe	182.8997	Zinc sulfate
	184.1033	Citric acid
	184.1139	Ammonium hydroxide
	184.1143	Ammonium sulfate
	184.1155	Bentonite
	184.1193	Calcium chloride
184.1212	Calcium pantothenate	
	184.1261	Copper sulfate

Algal Oil (*Chlorella*) Notification

Table of CFR Sections Referenced (Title 21—Food and Drugs)		
Part	Section §	Section Title
	184.1298	Ferric citrate
	184.1370	Inositol
	184.1400	Lecithin
	184.1443	Magnesium sulfate
	184.1461	Manganese sulfate
	184.1521	Monosodium phosphate derivatives of mono- and diglycerides
	184.1537	Nickel
	184.1555	Rapeseed oil
	184.1631	Potassium hydroxide
	184.1854	Sucrose
	184.1875	Thiamine hydrochloride
	184.1945	Vitamin B ₁₂
582—Substances generally recognized as safe	582.80	Trace minerals added to animal feeds

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**APPENDIX A: Intended Food-Uses and Use-Levels for
Solazyme's Algal Oil (*Chlorella*) in the United States**

Algal Oil (*Chlorella*) Notification

Table A-1 Summary of the Individual Proposed Food-Uses and Use-Levels for Algal Oil (<i>Chlorella</i>) in the U.S. (NHANES 2005-2006)				
Food Category	Proposed Food Uses	Use-Level (g/serving)	Serving Size¹ (g)	Use-Level (%)
Baked Goods and Baking Mixes	Biscuits	4.5	45	10
	Cakes	4.5	45	10
	Cookies and Brownies	4.5	45	10
	Crackers	3.0	28	10.71
	Muffins	4.5	45	10
	Pizza Dough	6.0	45	13.33
Beverages, Alcoholic	Algae Liqueur ²	0.25	14	1.79
Beverages and Beverage Bases	Algal Milk ²	15	240 ³	6.25
	Energy Drink Shots ²	1.5	85.13	1.76
	Nutritional and Meal Replacement Beverages (RTD)	17	240 ³	7.08
Confections and Frostings	Frostings and Icings	1.6	32	5
Fats and Oils	Frying, Cooking, and Specialty Oils	14	14	100
	Margarine and Margarine-Like Spreads	4.0	14	26
	Mayonnaise and Mayonnaise-Type Dressings	4.0	14	26
	Salad Dressings (regular and low calorie, excluding dry mixes)	4.0	14	26
Frozen Dairy Desserts and Mixes	Frozen Yogurt	11.3	113	10
Gelatins, Puddings, and Fillings	Dessert Toppings and Fillings	6.0	32	18.75
Grain Products and Pastas	Breakfast, Energy, and Meal Replacement Bars	4.5	45	10
		5.0	65	7.69
Milk Products	Milk-Based Nutritional and Meal Replacement Beverages (RTD)	17	240 ³	7.08
	Yogurt	11.3	113	10
Nut and Nut Products	Nut Butters and Spreads	3.0	32	9.38
Processed Fruits and Fruit Juices	Fruit-Based Smoothies	8.0	60	13.33
Snack Foods	Salty Snacks (pretzels, tortilla, and potato chips, etc.)	2.8	28	10
Soft Candy	Chocolate Candies	6.0	32	18.75
	Non-Chocolate Soft Candies	6.0	32	18.75
Soups and Soup Mixes	Soups (not powdered)	23	245 ³	9.39
Sweet Sauces, Toppings, and Syrups	Syrups and Sweet Sauces	3.2	32	10

RTD = Ready-to-drink

¹ Serving sizes were provided by Solazyme, unless otherwise indicated.

² No food codes were identified for these categories; therefore, surrogate codes were used to represent the food codes in these categories.

³ Serving sizes based on Reference Amounts Customarily Consumed (RACC) in the U.S. CFR (21 CFR § 101.12) (U.S. FDA, 2009b).

APPENDIX B: Chemical Analysis of Algal Oil (*Chlorella*)

Algal Oil (*Chlorella*) Notification

Non-consecutive lots of Solazyme's Algal oil (*Chlorella*) were analyzed for chemical parameters. The results are presented below in Table B-1.

Table B-1 Summary of the Chemical Product Analysis for 4 Lots of Algal Oil (<i>Chlorella</i>)					
Parameter	Specification	Lot Number			
		RBD128	RBD138	RBD265	CR227
Fat Quality					
Free Fatty Acid (%)	<1%	0.05	0.01	0.07	0.18
Unsaponifiable Matter (%)	<2.0%	0.53	0.6	0.26	0.53
Iodine Value	80 -110	86.4	94.0	85.2	89.1
Moisture & Volatiles (%)	<1%	0.03	0.001	0.02	0.1
Peroxide Value (meq/kg)	<5.0	<0.10	<0.10	<0.2	0.6
Residual hexane (ppm)	<1.0	<1.0	<1.0	<1.0	NA*
Heavy Metals					
Arsenic (ppm)	<0.2 ppm	<0.2	<0.2	<0.2	<0.2
Cadmium (ppm)	<0.1 ppm	<0.03	<0.03	<0.03	<0.03
Chromium (ppm)	<2 ppm	<0.02	<0.02	<0.02	<0.02
Lead (ppm)	<0.5 ppm	<0.2	<0.2	<0.2	<0.2
Mercury (ppm)	<0.2 ppm	<0.2	<0.2	<0.2	<0.2

NA= not applicable

*mechanically extracted

**APPENDIX C: Expert Panel Consensus Statement Regarding
the Generally Recognized as Safe (GRAS) Status of *Chlorella*
Oil for Use in Food**

Expert Panel Consensus Statement Regarding the Generally Recognized as Safe (GRAS) Status of *Chlorella* Oil for Use in Food

October 26, 2009

INTRODUCTION

At the request of Solazyme, Inc. (Solazyme), an Expert Panel (the "Panel") of independent scientists, qualified by their relevant national and international experience and scientific training to evaluate the safety of food ingredients, was specially convened to conduct a critical and comprehensive evaluation of the available pertinent data and information, and determine whether, under the conditions of intended use as a food ingredient, *Chlorella* oil would be Generally Recognized as Safe (GRAS) based on scientific procedures. The Panel consisted of the below-signed qualified scientific experts: Professor Joseph F. Borzelleca, Ph.D. (Virginia Commonwealth University School of Medicine), Professor Eric A. Johnson, Sc.D. (University of Wisconsin – Madison), and Professor Gary M. Williams, M.D. (New York Medical College).

The Panel, independently and collectively, critically examined a comprehensive package of scientific information and data compiled from the literature and other published sources through August 2009 by Cantox Health Sciences International. In addition, the Panel evaluated other information deemed appropriate or necessary, including data and information provided by Solazyme. The information evaluated by the Panel included details pertaining to the method of manufacture and product specifications, supporting analytical data, intended use-levels in specified foods, consumption estimates for all intended uses, and a comprehensive assessment of the available scientific literature pertaining to the safety of *Chlorella* oil.

Following independent, critical evaluation of such data and information, the Panel convened *via* teleconference on 1 October 2009 and unanimously concluded that the intended uses in traditional foods described herein for *Chlorella* oil, meeting appropriate food-grade specifications as described in the supporting dossier [Documentation Supporting the Evaluation of *Chlorella* Powder and Oil as Generally Recognized as Safe (GRAS) for Use in Food] and manufactured consistent with current Good Manufacturing Practice (cGMP), are GRAS based on scientific procedures. A summary of the basis for the Panel's conclusion is provided below.

SUMMARY AND BASIS FOR THE GRAS STATUS OF THE INTENDED USES OF CHLORELLA OIL

Solazyme's *Chlorella* oil is produced from the dried biomass of *Chlorella protothecoides* S106. *C. protothecoides* S106 is a species within the *Chlorella* genus. *Chlorella* are unicellular green algae belonging to the Division *Chlorophyta* that have been investigated for potential human consumption based upon their nutritional qualities.

Chlorella oil is manufactured in accordance with cGMP and all media components and processing aids are suitable for use in the manufacture of food. All media components and processing aids are removed through extensive purification processes. The manufacturing process for Solazyme's *Chlorella* oil begins with the fermentation of the *C. protothecoides* source organism. A pure, clonally isolated culture of *C. protothecoides* is initially used to prepare a master seed bank from which working seed vials are prepared. The Master and Working Cell Banks were characterized by molecular methods which showed they were genetically identical. For a production lot, a cryopreserved working seed vial is thawed and used to inoculate a flask, which is transferred into larger flasks at mid-log phase, and then to standard, industrial seed fermentors. Throughout the fermentation process, pH, temperature, and agitation and aeration rates are controlled, and glucose or sucrose and nutrient feeds are added. Using controlled fermentation conditions, either a low- or high-lipid containing *C. protothecoides* algal biomass can be produced. In this process, the *Chlorella* is first cultivated so as to produce a low lipid content, and lipid production is induced by limiting inorganic nitrogen during the latter part of the fermentation. Following completion of growth, the fermentation broth is harvested, concentrated, and dried. The oil is extracted from the dried biomass using mechanical or hexane extraction and may be further refined, bleached, and deodorized. If food-grade anti-oxidants are used, they are added to the oil prior to the packaging (*i.e.*, after extraction, or after refining, bleaching, and deodorizing of the oil).

The chemical specifications for *Chlorella* oil are presented in Table 1. *Chlorella* oil contains $\leq 1\%$ free fatty acids, $\leq 2.0\%$ unsaponifiable matter, $\leq 1.0\%$ moisture and volatiles, and has an iodine value between 80 and 110 and a peroxide value of ≤ 5.0 meq/kg fat.

Table 1 Chemical Specifications for <i>Chlorella</i> Oil		
Specification Parameter	Specification	Method
Free Fatty Acid	$\leq 1\%$	AOCS Ca 5a-40
Unsaponifiable Matter	$\leq 2.0\%$	AOCS Ca 6a-40
Moisture & Volatiles or Karl Fisher Moisture	$\leq 1.0\%$	AOCS Ca 2c-25; AOCS Ca 2d-25 (vacuum) AOCS Ca 2e-84
Iodine Value	80 -110	POS Internal
Peroxide Value	≤ 5.0 meq/kg	AOCS Cd 8-53
Heavy Metals		
Lead	<0.5 ppm	AOAC Cd 17-01 (modified)
Arsenic	<0.2 ppm	AOAC Cd 17-01 (modified)
Mercury	<0.2 ppm	AOAC Cd 17-01 (modified)
Cadmium	<0.1 ppm	AOAC Cd 17-01 (modified)
Chromium	<2 ppm	AOAC Cd 17-01 (modified)

Analysis of 2 non-consecutive lots of the crude *Chlorella* oil and the refined, bleached, and deodorized *Chlorella* oil indicate that the manufacturing process produces products that are consistent with the specifications, including limits set for heavy metals. The main fatty acids present in *Chlorella* oil are C18:1(as oleic acid), C18:2 and C16:0.

The oxidative rancidity of the oil in a sample of *Chlorella* high-fat powder prepared with 1 of 3 different antioxidants added at 2 different levels was assessed under ambient (23°C) and accelerated (40°C) storage conditions. The interim Day 34 results from samples stored at 40°C demonstrated that all samples tested were below the cut-off value for peroxide values of 5.0 meq/kg fat. A 12-month shelf life has been designed for *Chlorella* oil. Formal accelerated stability studies are ongoing.

Chlorella oil is intended to be used as an ingredient in baked goods and baking mixes, beverages (alcoholic), beverages and beverage bases, confections and frostings, fats and oils, frozen dairy desserts and mixes, gelatins, puddings, and fillings, grain products and pastas, milk products, nut and nut products, processed fruits and fruit juices, snack foods, soft candy, soups and soup mixes, and sweet sauces, toppings, and syrups at use-levels ranging from approximately 1.8 to 26%, with the exception of frying, cooking, and specialty oils at a use-level of 100%. The complete list of food-uses and use-levels is provided in Appendix 1.

The consumption of *Chlorella* oil from all intended food uses was estimated using the National Center for Health Statistics' (NCHS) National Health and Nutrition Examination Surveys (NHANES) for the years 2005-2006 (NHANES 2005-2006) (CDC, 2006, 2009), which provides the most appropriate data for evaluating food use and food consumption patterns in the U.S. Under the conditions of intended use of *Chlorella* oil, the total U.S. population mean all-user intake of *Chlorella* oil was 20.1 g/person/day on an absolute basis or 0.35 g/kg body weight/day on a body weight basis. The 90th percentile all-user intake of *Chlorella* oil from all proposed food-uses by the total population was 39.4 g/person/day, or 0.73 g/kg body weight/day on a body weight basis. Although *Chlorella* oil is intended for use in all food categories indicated, it is highly unlikely that *Chlorella* oil will be used in all food categories and all food-uses simultaneously due to the numerous other vegetable oils currently available on the market. In a more realistic scenario, *Chlorella* oil is expected to achieve levels of use similar to those of olive, canola, sunflower, or peanut oil, which have a daily *per capita* intake ranging from 0.25 (sunflower seed oil) to 11.7 (canola oil) g/person/day. The daily *per capita* intake of canola oil is approximately half the estimated total population mean all-user intake of *Chlorella* oil and approximately 3 times lower than the estimated total population 90th percentile all-user intake of *Chlorella* oil. Therefore, it is expected that the estimated intakes for *Chlorella* oil are substantial over-estimates of the anticipated actual consumption and that the actual levels of consumption will be more similar to the levels of olive, peanut, or sunflower oils.

Although the absorption, distribution, metabolism, and elimination of Solazyme's *Chlorella* oil have not been studied specifically, it is expected that the *Chlorella*-derived lipids will be

digested, absorbed, and metabolized through normal physiological processes (PDRNS, 2001), as the fatty acid composition of *Chlorella* oil is qualitatively similar to that of other vegetable oils, such as olive oil, canola oil, and soybean oil, and the lipids provided by *Chlorella* oil are constituents of the normal human diet.

C. protothecoides is a species of *Chlorella* that is compositionally similar to other *Chlorella* species such as *C. pyrenoidosa* and *C. vulgaris*. Although the chemical composition of *Chlorella* species is highly variable and is dependent upon the environmental conditions under which the *Chlorella* is grown (Milner, 1948), for example, the lipid content of *Chlorella pyrenoidosa* can vary from approximately 5 to 85%, on a dry weight basis, the composition of Solazyme's *Chlorella* high-fat powder (the source material for the oil) is within the range of results reported for other *Chlorella* species. Additionally, while the majority of the results presented were obtained from species used in research, the results reported by Kay (1991) were obtained from the analysis of a commercially-available *Chlorella* product (Sun Chlorella). The amino acid composition of Solazyme's *Chlorella* high-fat and high-protein powder (*i.e.*, the algal biomass) is similar to that reported for other *Chlorella* species. The main fatty acids present in *Chlorella* high-fat powder, *Chlorella* high-protein powder, *C. vulgaris*, and *C. pyrenoidosa* are C18:1(as oleic acid), C18:2, and C16:0; however, the quantitative distribution of each fatty acid varies between the products. The genomic sequence of *C. protothecoides* has been compared with that of other commercially-available *Chlorella* products (New Chapter *Chlorella regularis* 390 mg gel caps, Whole Foods Broken Cell Wall *Chlorella* 500 mg pressed tablets, and NutriBiotic CGF 500 mg pressed tablets) (Wolfe *et al.*, 1992; Solazyme, unpublished data). When the four sequences were compared, it was observed that the 23S ribosomal sequence for *C. protothecoides* clustered with that of the commercially-available *Chlorella*. Additionally, there was a high degree of 23S sequence identity between all *Chlorella* analyzed and that of *C. vulgaris*, signifying that *Chlorella* species are genetically similar. The data from the analyses of the chemical composition, amino acid composition, fatty acid composition, and of the genomic sequence of various species of *Chlorella* indicate that the *Chlorella* species are highly similar. The similarity permits the bridging of both toxicological and clinical studies conducted with differing *Chlorella* species to support the safety of *C. protothecoides*.

None of the algal or cyanobacterial toxins that have been identified in the published literature or mentioned in international food regulations [*i.e.*, amnesic shellfish poisoning toxins (Domoic acid), PSP toxins (N-sulfocarbamoyl toxins C1-4, B1, B2, decarbamoylgonyautoxins 1-4, gonyautoxins 1-4, decarbamoylsaxitoxin, saxitoxin, and neosaxitoxin), diarrhetic shellfish poisoning toxins (okadaic acid, dinophysistoxins, pectenotoxins, yessotoxins, azaspiracides, and gymnodimines), neurotoxic shellfish poisoning toxins (brevetoxins), and the cyanobacterial toxins (microcystins MC-RR, -LR, -YR, -LA, -LF, -LW, NOD, anatoxin-a, CYN, and BMAA)] were detected in the *Chlorella* high-fat powder (Luckas, 2008 [unpublished]; Day *et al.*, 2009).

Solazyme's high-fat powder from *C. protothecoides* containing 48% lipid was well-tolerated in a 28-day toxicity study (Day *et al.*, 2009). There were no signs of toxicity and no effect on body weight gains. Although sporadic statistically significant alterations in food consumption, food efficiency ratios, hematological and biochemical parameters, urinalyses, and mean and relative organ weights were noted among males and females, these changes were deemed to be toxicologically irrelevant due to the lack of a dose-response relationship, the fact that they occurred in only one sex, and the lack of supporting gross or microscopic alterations. There were no adverse changes in hematology, coagulation, clinical chemistry, or urinalysis parameters in male or female rats treated with the *Chlorella* high-fat powder containing 48% lipid, and there were no effects of treatment on organ weights or on the results of the histopathological analysis. Therefore, under the conditions of the study, the no-observed-adverse-effect level (NOAEL) was the highest concentration tested, 10% algal biomass in the diet, equating to 7,557 and 8,068 mg/kg body weight/day for males and females, respectively. This is equivalent to 3,627 and 3,873 mg/kg body weight/day of the oil assuming 48% composition of the algal biomass as the oil.

Several studies that evaluated the nutritional value and/or the safety of the dietary administration of *Chlorella* species and powders, including *C. pyrenoidosa* and *C. vulgaris*, in mice, rats, and piglets have been reported. These studies would likely have been conducted on *C. pyrenoidosa* and *C. vulgaris* material containing varying levels of lipid since it is well documented that the protein and lipid contents of *Chlorella* can vary considerably on a dry weight basis based upon differing growing conditions. Although the protein and lipid content can change considerably, comparative studies between species have shown a qualitative similarity in both the amino acid and fatty acid profiles. Based upon the understanding that the *C. pyrenoidosa* and *C. vulgaris* used in the studies would have contained lipids of similar composition to that of *C. protothecoides*, these studies can be used to corroborate the safety of the *Chlorella* oil.

The studies conducted on *C. pyrenoidosa* and *C. vulgaris* containing 8 to 12.8% lipids include a 45- and 110-day growth study conducted in rats in which gross and histopathological examinations were performed, a 10-week study investigating the effect of *C. vulgaris* on cadmium metabolism that also included 3 non-cadmium treated groups, a 9-week study to examine the effects of *C. vulgaris* on lipid metabolism in rats, a 34-day metabolism study conducted in rats, and 15- and 26-day growth studies conducted in piglets in which hematology and histology were examined (Lubitz, 1963; Khalawan *et al.*, 1980; Yap *et al.*, 1982; Lee *et al.*, 2008; Shim *et al.*, 2009). Although these studies were not conducted consistent with currently accepted toxicology guidelines, the results demonstrate that *Chlorella* species containing lipid provided in the diet support normal growth, are generally well-tolerated, and do not produce any evidence of overt toxicity. Sporadic histological abnormalities were observed in the pancreas and salivary glands of male CD rats provided *Chlorella* 71105 (lipid content not reported) in the diet at a concentration of 21% (~21 to 23 g/kg body weight/day) or 20.5% (~20.5 g/kg body

weight/day) with 0.2% methionine for periods of 110 or 45 days; however, not all of the rats were examined histologically, the observed abnormalities were not present in all rats examined, there was no evidence of a dose-response, and the authors noted that “the abnormalities discovered may be artifacts” (Lubitz, 1963). Liver abnormalities (yellow or fatty liver) were reported in 2 male CD rats that received approximately 139 g *Chlorella* 71105/kg body weight/day for a period of 37 days; however, this level of *Chlorella* in the diet also had a possible growth-retarding effect and the authors concluded that “the liver abnormality (a yellow or fatty liver) could be a secondary effect of the growth retardation” (Lubitz, 1963). Male Wistar rats that were provided *C. vulgaris* containing 12.8% lipids in the diet at a level of 5 or 10% (equivalent to approximately 5,000 and 10,000 mg/kg body weight/day, respectively) for 9 weeks had a decreased liver weight relative to body weight compared to the control group (Lee *et al.*, 2008). Although histopathological examinations were not performed, the decrease in relative liver weight was not accompanied by any significant changes in serum aspartate aminotransferase (AST) or alanine transferase (ALT) activities or total protein or bilirubin concentrations. There were no significant differences in liver or kidney weights, serum AST, ALT, or creatinine, urinary creatinine, or creatinine clearance between male CD rats administered *C. vulgaris* containing 12.8% lipids at a concentration of 3 or 5% (equivalent to approximately 3,000 and 5,000 mg/kg body weight/day, respectively) for 10 weeks compared to control rats (Shim *et al.*, 2009). No signs of clinical toxicity, and no significant differences in hematological or histological parameters or liver weights were observed following the provision of *Chlorella* sp. (lipid content not reported) in the diet of Yorkshire piglets at a level of 13.81% from Days 4 to 15 or Days 8 to 26 of age (equivalent to approximately 600 and 500 mg/kg body weight/day, respectively) compared to controls (Yap *et al.*, 1982). Similarly, no signs of clinical toxicity, no behavioral changes, and no toxicologically relevant adverse effects upon post-mortem examination were observed in female Harvard rats following the consumption of approximately 9 g *C. pyrenoidosa* (containing 8% lipids)/kg body weight/day in the diet for 34 days compared to controls (Khalawan *et al.*, 1980). No effects on final body weights, body weight gains, food intake, or food efficiency ratios were observed in rats administered diets supplemented with *Chlorella* species (containing 8 to 14.4% lipids) at concentrations between 5 and 92% (approximately 7,200 to up to 23,157 mg/kg body weight/day [U.S. FDA, 1993]) for durations between 1 and 30 weeks (Lubitz, 1963; Wang *et al.*, 1979, 1980; Khalawan *et al.*, 1980; Saleh *et al.*, 1985; Sano *et al.*, 1988; Herrero *et al.*, 1993; Shibata *et al.*, 2001; Cherng and Shih, 2005). Mice, hamsters, and piglets administered *Chlorella* species, including *Chlorella* sp. (lipid content not reported), *C. vulgaris* (lipid content not reported), and *C. pyrenoidosa* (containing 13% lipids), in their diets at levels of 1, 7.2, or 13.81% (equivalent to approximately 1,560, 8,640, and 492 to 614 mg/kg body weight/day) for 10 or 8 weeks, or 11 to 18 days, respectively, also displayed no biologically significant adverse effects on growth or food intake throughout the study period (Yap *et al.*, 1982; Chovančíková and Šimek, 2001; Cherng and Shih, 2005). Although not consistently measured in the above studies, no toxicologically significant adverse effects were noted following hematological or biochemical analyses.

The results of an unpublished 2-week toxicity study further support the safety of Solazyme's *Chlorella* oil (Krishnaswamy, 2000). No toxicologically significant differences in body weights, food intake, behavior, neurological signs, serum AST or ALT, urinalysis parameters, or gross necropsy or histopathological examinations were reported following the administration of 125, 250, 500, 1,000, or 2,000 mg *C. vulgaris* E25 (containing 10% lipids)/kg body weight/day by gavage to Fischer 344 rats in an escalating dose-pattern compared to controls. Under the conditions of the study, it can be determined that the NOAEL was 2,000 mg/kg body weight/day, the highest dose tested.

The absence of adverse reproductive effects from the consumption of *Chlorella* was demonstrated in a three-generation reproduction study conducted with Fzt:DU mice (Janczyk *et al.*, 2006). The provision of a 1.0% spray-dried *C. vulgaris* (containing 8.1% lipids) supplemented diet to mice over 3 generations did not have any effects on adult body weights at age 42 or 63 days, weight of litters at age 0, 10, or 21 days, mean weight of litter mates per litter at age 0, 10, or 21 days, weight of fetuses on Day 16 or 18 of gestation, number of live mouse pups per litter, survival rate of pups from birth to weaning, or number of live, dead, and absorbed fetuses, or corpora lutea in any generation compared to control mice.

No traditional genotoxicity or 2-year carcinogenicity studies were identified in the literature. The effects of *Chlorella* sp. on tumor growth have been investigated in mice and rats. *C. vulgaris* dried powder (lipid content not reported) or its acetone extract administered in the diet for up to 57 days at levels of up to 15 g/kg body weight/day did not promote the growth of subcutaneously inoculated 3-methylcholanthrene-induced tumor cells in CDF1 mice (Tanaka *et al.*, 1990). Additionally, the authors reported that dietary administration of *C. vulgaris* dried powder resulted in no serious side effects including decreases in body weights or other wasting syndromes. Dietary *C. pyrenoidosa* (comprised of 11.2% lipids) at a concentration of 10% (equivalent to approximately 6,960 mg/kg body weight/day) administered for 59 days was observed to have an inhibitory effect on hepatocarcinogenesis in male F344/DuCrj rats initiated and/or promoted with diethylnitrosamine and 2-amino-3,8-dimethylimidazo[4,5-f] quinoxaline, respectively (Takekoshi *et al.*, 2005). Bone marrow colony formation was significantly increased and survival was prolonged in male BALB/c mice inoculated with Erlich ascites tumor following the administration of *C. vulgaris* extract by gavage for 5 days at doses of 50, 100, or 200 mg/kg body weight/day compared to placebo tumor-bearing mice (Justo *et al.*, 2001).

The consumption of *Chlorella* species containing 11 to 19% lipids by humans was reported to be well-tolerated in a number of studies in which the beneficial effects of the algae on the immune system, hypertension, fibromyalgia syndrome, ulcerative colitis, and glioma (primary brain tumors) were investigated, as well as in studies where *Chlorella* replaced dietary high-quality protein sources such as fish, egg, and soy as the principle source of nitrogen consumption (Dam *et al.*, 1965; Lee *et al.*, 1967; Merchant *et al.*, 1990, 2000, 2002; Merchant and Andre, 2001; Halperin *et al.*, 2003). The only adverse effects reported following the

consumption of up to 90 g of *Chlorella* species for periods of up to 2 years were feelings of fatigue following the consumption of 200 mg *C. pyrenoidosa* (lipid content not reported) per day for a period of 28 days (Halperin *et al.*, 2003) and symptoms of gastrointestinal upset, nausea, and fever during the first week of treatment with 20 g of *C. pyrenoidosa* (containing approximately 11% lipids) and 150 mL of a liquid *C. pyrenoidosa* extract (Merchant *et al.*, 1990). The symptoms of gastrointestinal upset were noted to generally subside over the rest of 2-year study period. In a 26-day study in which 5 healthy males (aged 18 to 23 years) were provided a mixture of *Chlorella* and *Scenedesmus* (ratio not reported) containing 19% lipids in gingerbread, chocolate cake, chocolate cookies, and milk in increasing amounts from 10 to up to 500 g/day, the authors concluded that the “algae in amounts up to 100 g/man/day can be well-tolerated at least for a short time” (Powell *et al.*, 1961). When provided at levels greater than 100 g/day, the volunteers had difficulty digesting the test items and experienced abdominal distention, associated with increased erucation and flatulence. Nausea, mild abdominal cramping pain, headache, malaise, and hard bulky stools were reported by the subjects when the level of algae consumed reached 500 g algae/day. No abnormalities were reported in physical examinations other than those associated with the gastrointestinal tract, and hematology, urinalysis, and liver function tests were all within normal limits. Similarly, no adverse effects or significant differences in hematology, clinical chemistry, or urinalysis parameters were reported to occur in subjects with fibromyalgia syndrome, ulcerative colitis, mild to moderate hypertension, or malignant gliomas following the consumption of up to 20 g *Chlorella* (containing approximately 11% lipids) and 150 mL *Chlorella* extract for periods of up to 2 years (Merchant *et al.*, 1990, 2000, 2002; Merchant and Andre, 2001).

Photosensitive dermatitis is the only significant adverse effect associated with the consumption of *Chlorella* species in humans. This effect was determined to result from the presence of pheophorbide a, a breakdown product of chlorophyll a (Jitsukawa *et al.*, 1984; Jassby, 1988). An investigation by Tokyo Bureau of Metropolitan Health revealed that 23 cases of photosensitive dermatitis that occurred between June 1976 and June 1977 occurred in persons consuming a specific brand of *Chlorella*, “Kenbi *Chlorella*” (Jitsukawa *et al.*, 1984). Furthermore, only *Chlorella* products produced between April 1976 and April 1977 were reported to cause photosensitive dermatitis, which coincided with a change in the manufacturing process during the drying process (moistening the *Chlorella* powder with water and ethanol followed by drying at 90°C for 30 minutes). As the enzyme responsible for pheophorbide-a production, chlorophyllase, is reported to have a high activity at 80°C but no activity at 100°C, the change in manufacturing was determined to cause the production of pheophorbide-a. In 1981, the Japanese Public Health Ministry recommended that the level of pheophorbide-a in algae preparations be restricted to less than 1.2 mg/g (Becker, 1994). The analysis of 2 non-consecutive lots of Solazyme’s *Chlorella* powder (1 high-protein and 1 high-fat) demonstrated that the level of pheophorbide a in Solazyme’s *Chlorella* powder (not detected to 0.0334 mg/g) is below the limit established by the Japanese Public Health Ministry. The results of the studies conducted in humans with the oral administration of various species of *Chlorella* do not indicate

any potential for toxicity or cause for concern resulting from the consumption of Solazyme's *Chlorella* oil under the conditions of intended use. *Chlorella* also has a history of consumption in the U.S. in the form of dietary supplements, as is demonstrated by its inclusion in the United Natural Products Alliance (UNPA) (nee Utah Natural Products Alliance) 'old dietary ingredient list'. Inclusion on the list indicates that *Chlorella* was sold and in the market place prior to the implementation of the *Dietary Supplement Health and Education Act of 1994* (DSHEA, 1994). The number of years that *Chlorella* dietary supplement products have been available on the U.S. market, the lack of any reference to *Chlorella* in the U.S. adverse event reporting program, and the few reports in the Canadian adverse reaction database indicate that *Chlorella* supplements are well-tolerated and without cause for concern to human health.

Limited data regarding the potential allergenicity of *Chlorella* species were identified in the literature (Tiberg *et al.*, 1990a,b, 1995). Unlike, the biomass, the *Chlorella* oil will contain limited or no protein. However, even knowing that the protein content of the oil will be extremely low, the results of these studies indicate that proteins of *C. vulgaris*, *C. saccharophila*, or *C. homosphaera* do not have significant allergenic potential, even in atopic individuals. It is important to note that no reports of allergy were identified in the scientific literature following consumption of *Chlorella*. The long history of safe consumption of *Chlorella* products strongly supports the lack of allergenic potential.

The weight of the scientific evidence presented herein from the Solazyme study specifically and the other corroborative safety studies indicates that the intended uses of *Chlorella* oil, meeting appropriate food-grade specifications and manufactured in accordance with cGMP, are safe and suitable. The data and information summarized in this report demonstrate that the intended uses of the *Chlorella* oil would be GRAS based on scientific procedures.

CONCLUSION

We, the Expert Panel, have independently and collectively, critically evaluated the data and information summarized above and conclude that the intended uses of *Chlorella* oil, meeting appropriate food-grade specifications presented in the supporting dossier [Documentation Supporting the Evaluation of *Chlorella* Powder and Oil as Generally Recognized as Safe (GRAS) for Use in Food] and produced consistent with current good manufacturing practice (cGMP), are safe and suitable.

We further conclude that the intended uses of *Chlorella* oil, meeting appropriate food-grade specifications presented in the supporting dossier and produced consistent with cGMP, are GRAS based on scientific procedures.

It is our opinion that other qualified experts would concur with these conclusions.

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Appendix 1

Table A-1 Summary of the Individual Proposed Food-Uses and Use-Levels for *Chlorella* Oil in the U.S. (NHANES 2005-2006)

Food Category	Proposed Food Uses	Use-Level (g/serving)	Serving Size ¹ (g)	Use-Level (%)
Baked Goods and Baking Mixes	Biscuits	4.5	45	10
	Cakes	4.5	45	10
	Cookies and Brownies	4.5	45	10
	Crackers	3.0	28	10.71
	Muffins	4.5	45	10
	Pizza Dough	6.0	45	13.33
Beverages, Alcoholic	Algae Liqueur ²	0.25	14	1.79
Beverages and Beverage Bases	Algal Milk ²	15	240 ³	6.25
	Energy Drink Shots ²	1.5	85.13	1.76
	Nutritional and Meal Replacement Beverages (RTD)	17	240 ³	7.08
Confections and Frostings	Frostings and Icings	1.6	32	5
Fats and Oils	Frying, Cooking, and Specialty Oils	14	14	100
	Margarine and Margarine-Like Spreads	4.0	14	26
	Mayonnaise and Mayonnaise-Type Dressings	4.0	14	26
	Salad Dressings (regular and low calorie, excluding dry mixes)	4.0	14	26
Frozen Dairy Desserts and Mixes	Frozen Yogurt	11.3	113	10
Gelatins, Puddings, and Fillings	Dessert Toppings and Fillings	6.0	32	18.75
Grain Products and Pastas	Breakfast, Energy, and Meal Replacement Bars	4.5	45	10
		5.0	65	7.69
Milk Products	Milk-Based Nutritional and Meal Replacement Beverages (RTD)	17	240 ³	7.08
	Yogurt	11.3	113	10
Nut and Nut Products	Nut Butters and Spreads	3.0	32	9.38
Processed Fruits and Fruit Juices	Fruit-Based Smoothies	8.0	60	13.33
Snack Foods	Salty Snacks (pretzels, tortilla, and potato chips, etc.)	2.8	28	10
Soft Candy	Chocolate Candies	6.0	32	18.75
	Non-Chocolate Soft Candies	6.0	32	18.75

Table A-1 Summary of the Individual Proposed Food-Uses and Use-Levels for *Chlorella* Oil in the U.S. (NHANES 2005-2006)

Food Category	Proposed Food Uses	Use-Level (g/serving)	Serving Size ¹ (g)	Use-Level (%)
Soups and Soup Mixes	Soups (not powdered)	23	245 ³	9.39
Sweet Sauces, Toppings, and Syrups	Syrups and Sweet Sauces	3.2	32	10

RTD = Ready-to-drink

¹ Serving sizes were provided by Solazyme, unless otherwise indicated.

² No food codes were identified for these categories; therefore, surrogate codes were used to represent the food codes in these categories.

³ Serving sizes based on Reference Amounts Customarily Consumed (RACC) in the U.S. CFR (21 CFR § 101.12) (U.S. FDA, 2009).

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