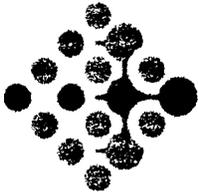


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Original Submission

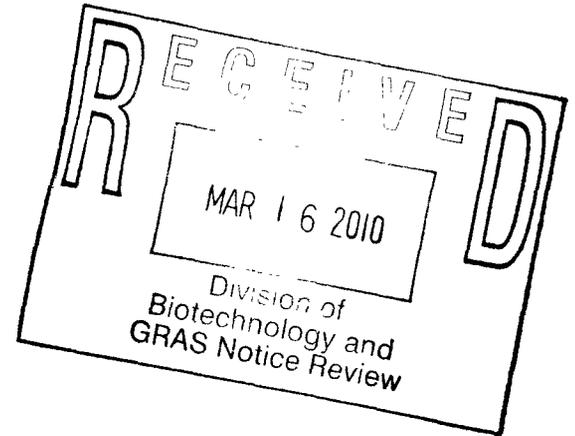
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SENT VIA FEDEX

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 flour (*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 flour (*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 flour (*Chlorella*) ingredient under the intended conditions of use, also are enclosed.

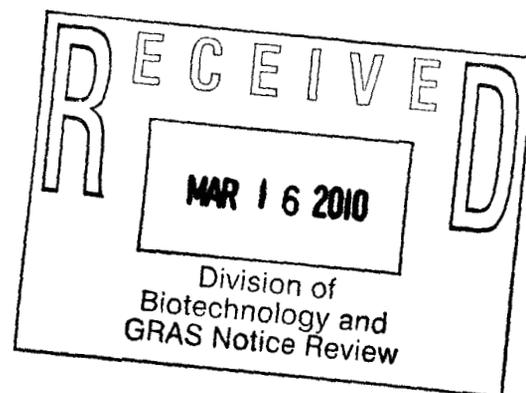
I trust that the enclosed Notice is acceptable. Should you have any questions or concerns regarding this GRAS Notice, please do not hesitate to contact me at any point during the review process so that we may provide a response in a timely manner.

Sincerely, (b) (6)

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

Enclosure

000004



ALGAL FLOUR (*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

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Table of Contents

	Page
I. GRAS Exemption Claim	3
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)]	3
I.B. Name and Address of Notifier	3
I.C. Common Name of the Notified Substance	3
I.D. Conditions of Intended Use in Food	4
I.E. Basis for the GRAS Determination	4
I.F. Availability of Information	4
II. Detailed Information About the Identity of the Substance	4
II.A. Identity	4
II.B. Method of Manufacture	5
II.C. Specifications for Food Grade Material	8
II.D. Stability of Algal Flour (<i>Chlorella</i>)	9
III. Self-Limiting Levels of Use	10
IV. Basis for GRAS Determination	10
IV.A. Current Regulatory Status and History of Use	11
IV.B. Estimated Intake of Algal Flour (<i>Chlorella</i>)	11
IV.C. Composition of <i>Chlorella</i> Species	13
IV.D. Metabolic Fate and Kinetics	14
IV.E. Toxicity Studies	14
(i) Acute Toxicity Studies	14
(ii) Subchronic Toxicity Studies	14
(iii) Reproductive and Teratogenic Toxicity Studies	27
(iv) Mutagenicity and Genotoxicity Studies	27
(v) Carcinogenicity Studies	28
(vi) Human Studies	28
IV.F. Algal Cyanobacterial Toxin and Pheophorbide Analysis	38
IV.G. Allergy	38
IV.H. Summary and Basis for GRAS	38
V. Conclusion	41
VI. References	41

Algal Flour (*Chlorella*) Notification

List of Appendices

- APPENDIX A Intended Food-Uses and Use-Levels for Solazyme's Algal Flour (*Chlorella*) in the United States
- APPENDIX B Chemical and Microbiological Batch Analyses of Algal High-Protein Flour (*Chlorella*) and Algal High-Lipid Flour (*Chlorella*)
- APPENDIX C Expert Panel Consensus Statement Regarding the Generally Recognized as Safe (GRAS) Status of *Chlorella* Powder (High-Fat and High-Protein) for Use in Food

List of Tables

Table II.B-1	Raw Materials in the Growth Medium, Fermentation Process, and Production of Flour and Antioxidants in the Manufacture of Algal Flour (<i>Chlorella</i>)	5
Table II.C-1	Chemical and Microbiological Specifications for Algal High-Lipid and High-Protein Flour (<i>Chlorella</i>)	9
Table II.D-1	148-Day Interim Results of An Ongoing Shelf-Life Study on Algal High-Lipid Flour (<i>Chlorella</i>)	10
Table IV.B-1	Summary of the Estimated Daily Intake of Algal Flour (<i>Chlorella</i>) from All Proposed Food-Uses in the U.S. by Population Group (2005-2006 NHANES Data).....	12
Table IV.B-2	Summary of the Estimated Daily Per Kilogram Body Weight Intake of Algal Flour (<i>Chlorella</i>) from All Proposed Food-Uses in the U.S. by Population Group (2005-2006 NHANES Data)	13
Table IV.C-1	Summary of Subchronic Toxicity Studies on <i>Chlorella</i> sp.	18
Table IV.C-2	Summary of Human Safety Studies on <i>Chlorella</i> Species	30
Table A-1	Summary of the Individual Proposed Food-Uses and Use-Levels for Algal Flour (<i>Chlorella</i>) in the U.S. (NHANES 2005-2006)	A-1
Table B-1	Summary of the Chemical and Microbiological Product Analysis for 3 Lots of Algal High-Protein Flour (<i>Chlorella</i>).....	B-1
Table B-2	Summary of the Chemical and Microbiological Product Analysis for 3 Lots of Algal High-Lipid Flour (<i>Chlorella</i>).....	B-2

Algal Flour (*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, high-lipid and high-protein Algal flour (*Chlorella*), produced from the dried biomass of *Chlorella protothecoides* S106, have 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 flour (*Chlorella*) (high-lipid and high-protein) under the conditions of its intended use in food. Therefore, the use of Solazyme's high-lipid and high-protein Algal flour (*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 flour (*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* Powder (High-Fat and High-Protein) for Use in Food". Note that Solazyme's Algal flour (*Chlorella*) (high-lipid and high-protein) – the ingredient that is the subject of this Notification – is referred to as *Chlorella* powder (high-fat and high-protein) throughout the Expert Panel Consensus Statement in Appendix C.

Algal Flour (*Chlorella*) Notification

I.D. Conditions of Intended Use in Food

Solazyme intends to market 2 Algal flour (*Chlorella*) products, a high-lipid and a high-protein flour, as food ingredients in the United States under the proposed food uses as described in Table A-1 (Appendix A). Both Algal flour (*Chlorella*) products (high-lipid and high-protein) are intended to be used in the same food categories at the same use levels, which range from 3.5 to 50%. It is important to note that an individual food product is intended to contain either the high-lipid or the high-protein flour, and not both flours at the same time.

I.E. Basis for the GRAS Determination

Pursuant to 21 CFR § 170.30, high-lipid and high-protein Algal flour (*Chlorella*) have 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 flour (*Chlorella*) (high-lipid and high-protein) 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 flour (*Chlorella*) (high-lipid and high-protein) 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 high-lipid and high-protein Algal flour (*Chlorella*) are produced from the dried biomass of *Chlorella protothecoides* S106. *C. protothecoides* S106 is a species within the *Chlorella* genus.

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

Chemical Name: *Chlorella protothecoides* S106 high-lipid and high-protein flour

Algal Flour (*Chlorella*) Notification

Chemical Abstracts Service (CAS) Number: Not applicable

Empirical Formula: Not Applicable

Molecular Weight: Not Applicable

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

The high-lipid and high-protein Algal flours (*Chlorella*) are produced from the dried biomass of a pure culture of *C. protothecoides* S106. All raw materials used in the growth medium, fermentation process, and production of flour, 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 Flour and Antioxidants in the Manufacture of Algal Flour (<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).

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Algal Flour (*Chlorella*) Notification

Table II.B-1 Raw Materials in the Growth Medium, Fermentation Process, and Production of Flour and Antioxidants in the Manufacture of Algal Flour (<i>Chlorella</i>)		
Material	Use	Regulatory Status
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).
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 limitations other 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).

Algal Flour (*Chlorella*) Notification

Table II.B-1 Raw Materials in the Growth Medium, Fermentation Process, and Production of Flour and Antioxidants in the Manufacture of Algal Flour (<i>Chlorella</i>)		
Material	Use	Regulatory Status
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.
Flour Production		
Potable Water	Wash the fermentation broth	Water which meets the standards prescribed in the EPA's Primary Drinking Water Regulations as set forth in 40 CFR § 141.
Antioxidants		
Fortium® Brand MTD10 Liquid Antioxidant (consists of canola oil and natural mixed tocopherols)	Antioxidant, stabilize the flour	Canola oil is affirmed as GRAS as a direct food substance (21 CFR § 184.1555) and tocopherols are GRAS as chemical preservatives (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 flour	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 flour	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 flour	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; EPA = U.S. Environmental Protection Agency; FDA = U.S. Food and Drug Administration; GRAS = generally recognized as safe; USP = United States Pharmacopeia. CFR = U.S. Code of Federal Regulations (U.S. EPA, 2009; U.S. FDA, 2009b)

The source strain for Algal flour (*Chlorella*) (high-lipid and high-protein) 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

Algal Flour (*Chlorella*) Notification

ribosomal DNA sequences also demonstrated 100% identity to the 23S reference sequence for the original S106 isolate.

The manufacturing process for Solazyme's Algal flour (*Chlorella*) (high-lipid and high-protein) 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. Lipid production is induced by limiting inorganic nitrogen during the latter part of the fermentation process. Following completion of growth, the fermentation broth is harvested, concentrated, optionally washed and/or disrupted, and then dried and packaged. Food-grade antioxidants may be added following the wash stage.

II.C. Specifications for Food Grade Material

Algal flour (*Chlorella*) (high-lipid and high-protein) is produced in accordance with current good manufacturing practice (cGMP) and food grade chemical and microbiological specifications have been established for the final products by Solazyme to ensure consistent, safe products. The chemical and microbiological specifications for high-lipid and high-protein Algal flours (*Chlorella*) are presented in Table II.C-1. Algal high-lipid flour (*Chlorella*) is composed of 40 to 70% lipid, 2 to 15% protein, and 10 to 50% fiber, with a moisture content of 10% or less. Algal high-protein flour (*Chlorella*) is composed of 40 to 70% protein, 5 to 25% lipid, and 5 to 25% fiber, with a moisture content of 10% or less. Analysis of 3 non-consecutive lots of Algal high-protein flour (*Chlorella*) and 3 non-consecutive lots of Algal high-lipid flour (*Chlorella*) indicate that the manufacturing process produces products that are consistent with their respective specifications, including limits set for heavy metals and microbes. The complete analyses of these batches are presented in Tables B-1 and B-2 (Appendix B). All analytical procedures are conducted using standard validated methodologies [*i.e.*, Association of Official Analytical Chemists (AOAC), United States Pharmacopeia (USP), National Formulary (NF), and the U.S. FDA Bacteriological Analytical Manual (FDA-BAM)].

Algal Flour (*Chlorella*) Notification

Table II.C-1 Chemical and Microbiological Specifications for Algal High-Lipid and High-Protein Flour (<i>Chlorella</i>)			
Specification Parameter	Specification		Method
	High-Lipid	High-Protein	
<i>Proximate</i>			
Moisture Content	≤10%	≤10%	AOAC 930.15
Fiber Content	10 to 50%	5 to 25%	AOAC 991.43
Ash Content	<10%	<10%	AOAC 942.05
Protein Content	2 to 15%	40 to 70%	AOAC 990.03
Lipid Content	40 to 70%	5 to 25%	AOAC 954.02
Sucrose	0 to 10%	0 to 10%	AOAC 980.13
<i>Heavy Metals</i>			
Lead	<0.5 ppm	<0.5 ppm	ICP-MS/AOAC 984.27 (microwave) or GLI Procedure ME-30
Arsenic	<0.2 ppm	<0.2 ppm	ICP-MS/AOAC 984.27 (microwave) or GLI Procedure ME-30
Mercury	<0.1 ppm	<0.1 ppm	ICP-MS/AOAC 984.27 (microwave) or GLI Procedure E80-3
Cadmium	<0.1 ppm	<0.1 ppm	ICP-MS/AOAC 984.27 (microwave) or GLI Procedure ME-30
Chromium	<2 ppm	<2 ppm	AOAC 975.03 or AOAC 984.27 or GLI Procedure ME-30
<i>Microbiological Limits</i>			
Aerobic Plate Count	<10,000 cfu/g	<10,000 cfu/g	AOAC 966.23
<i>E. coli</i>	Negative in 25 g	Negative in 25 g	USP31, NF26, 2008
<i>Staphylococci</i>	Negative in 25 g	Negative in 25 g	USP31, NF26, 2008
<i>Salmonella</i>	Negative in 25 g	Negative in 25 g	AOAC 2004.03
Yeast	<100 cfu/g	<100 cfu/g	FDA-BAM, 7 th ed.
Mold	<100 cfu/g	<100 cfu/g	FDA-BAM, 7 th ed.

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

The oxidative rancidity of the oil in samples of Algal high-lipid flour (*Chlorella*) was used to assess the stability of Algal flour (*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 SaffTest® System, manufactured by MP Biomedicals (Solon, OH). The interim results from samples stored at 40°C 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 flour (*Chlorella*) (high-lipid and high-protein). Formal accelerated stability studies are ongoing.

Algal Flour (*Chlorella*) Notification

Table II.D-1 148-Day Interim Results of An Ongoing Shelf-Life Study on Algal High-Lipid Flour (*Chlorella*)

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 flour (*Chlorella*) (high-lipid and high-protein), no self-limiting use levels are expected.

IV. Basis for GRAS Determination

The determination that high-lipid and high-protein Algal flour (*Chlorella*) are GRAS is on the basis of scientific procedures. The safety of Algal flour (*Chlorella*) (high-lipid and high-protein) 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, the results of a published product specific pre-clinical toxicity study, 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 flour (*Chlorella*) (high-lipid and high-protein) as a food ingredient, who concluded that the aforementioned proposed uses of Algal flour (*Chlorella*) (high-lipid and high-protein) 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 Flour (*Chlorella*)

Both Algal flour (*Chlorella*) products (high-lipid and high-protein) are intended to be used in the same food categories at the same use-levels; however, an individual food product is intended to contain either the high-lipid or the high-protein product, and not both products at the same time. Thus, the estimated intakes of Algal high-lipid and high-protein flours (*Chlorella*) were calculated as a single Algal flour (*Chlorella*) product. Both Algal flour (*Chlorella*) products are intended to be used as ingredients in baked goods and baking mixes, beverages and beverage bases, dairy product analogs, egg products, fats and oils, gravies and sauces, processed vegetables and vegetable juices, and soups and soup mixes at use-levels ranging from 3.5 to 50%. The complete list of food-uses and use-levels is provided in Appendix A. The consumption of Algal flour (*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).

Algal Flour (*Chlorella*) Notification

Approximately 91.5% of the total U.S. population was identified as potential consumers of Algal flour (*Chlorella*) from the proposed food-uses (7,710 actual users identified). Consumption of these types of foods by the total U.S. population resulted in an estimated mean all-user Algal flour (*Chlorella*) intake of 13.8 g/person/day on an absolute basis or 0.23 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 flour (*Chlorella*) from all proposed food-uses by the total population was observed to be 29.1 g/person/day, or 0.49 g/kg body weight/day on a body weight basis. On an individual population basis, the greatest mean all-user intake of Algal flour (*Chlorella*) on an absolute basis was observed to occur in male adults, at 17.0 g/person/day (Table IV.B-1). Infants displayed the lowest mean all-user intake of Algal flour (*Chlorella*) on an absolute basis, with a value of 4.9 g/person/day. On a body weight basis, the mean all-user intake of Algal flour (*Chlorella*) was highest in children, with a value of 0.41 g/kg body weight/day (Table IV.B-2). The lowest mean all-user intake on a per kilogram body weight basis was observed to occur in female adults, with a value of 0.17 g/kg body weight/day.

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	62.4	613	3.3	8.7	4.9	9.9
Children	3 to 11	97.4	1,408	10.6	22.2	10.9	22.4
Female Teenagers	12 to 19	93.3	928	11.1	23.9	12.0	23.9
Male Teenagers	12 to 19	96.4	907	16.2	34.8	16.9	35.8
Female Adults	20 and up	95.1	2,047	11.8	26.6	12.5	27.1
Male Adults	20 and up	94.5	1,807	16.1	35.2	17.0	36.1
Total Population	All Ages	91.5	7,710	13.0	28.3	13.8	29.1

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	62.4	613	0.27	0.67	0.40	0.84
Children	3 to 11	97.4	1,408	0.40	0.87	0.41	0.88
Female Teenagers	12 to 19	93.3	928	0.19	0.40	0.20	0.42
Male Teenagers	12 to 19	96.4	907	0.25	0.59	0.26	0.60
Female Adults	20 and up	95.1	2,047	0.17	0.35	0.17	0.36
Male Adults	20 and up	94.5	1,807	0.19	0.40	0.20	0.41
Total Population	All Ages	91.5	7,710	0.21	0.47	0.23	0.49

Solazyme has noted that the methodology used to estimate the consumption of Algal flour (*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 flour (*Chlorella*) in the U.S. population.

Solazyme also has noted that although relatively high intake estimates of 9.9 g Algal flour (*Chlorella*)/day (0.84 g Algal flour (*Chlorella*)/kg body weight/day) were obtained in infants on an all-user basis from all proposed food-uses of Algal flour (*Chlorella*) at the 90th percentile, it should be stressed that the specified food-uses for Algal flour (*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 flour (*Chlorella*)-containing food products is expected to be limited. Therefore, although an estimate of the consumption of Algal flour (*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 flour (*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

Algal Flour (*Chlorella*) Notification

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 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 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*.

IV.D. Metabolic Fate and Kinetics

Although the absorption, distribution, metabolism, and elimination of Solazyme's Algal flours (*Chlorella*) have not been studied specifically, it is expected that the *Chlorella*-derived proteins, lipids, and carbohydrates will be digested, absorbed, and metabolized through normal physiological processes (PDRNS, 2001), as the macronutrients contained in the flour are constituents of the normal human diet

IV.E. 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* 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

Algal Flour (*Chlorella*) Notification

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 Algal high-lipid flour (*Chlorella*), 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.

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 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 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 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* 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).

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)

Algal Flour (*Chlorella*) Notification

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* 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. 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 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*/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 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) to mice for 10 weeks, diets containing 7.2% *C. pyrenoidosa* (approximately 8,640 mg/kg body weight/day) to hamsters for 8 weeks, or diets containing 13.81% *Chlorella* sp. 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 flour (*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/kg body weight/day by gavage to Fischer 344

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).

Algal Flour (*Chlorella*) Notification

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.C-1.

Algal Flour (*Chlorella*) Notification

Table IV.C-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>Chlorella protothecoides</i>						
Rat (Hsd:Sprague-Dawley SD; 10/sex/group)	28 days	<i>C. protothecoides</i> [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

000023

Algal Flour (*Chlorella*) Notification

Table IV.C-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> [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 [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 [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.	
Rat (CD; 10M/group)	45 days	T1: <i>Chlorella</i> 71105 [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.	

000024

Algal Flour (*Chlorella*) Notification

Table IV.C-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
		T2: <i>Chlorella</i> 71105 + 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. [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. extract via 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
Rat (Sprague-Dawley; 3 to 7F/group)	Exp 1: 10 weeks	<i>Chlorella</i> sp. [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. [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.	

000025

Algal Flour (*Chlorella*) Notification

Table IV.C-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
	Exp 3: 6 weeks	T1: Basal diet T2: <i>Chlorella</i> sp. [provided in feed at a concentration of 5%] T3: Ethionine [provided in feed at a concentration of 0.25%] T4: <i>Chlorella</i> sp. + ethionine [provided in feed at a concentration of 5% and 0.25%, respectively]	0 or 5,000 ^c	<ul style="list-style-type: none"> Body weight 	T2: ↑ body weight gain (significance not reported)	
Piglet [Yorkshire; M, F, 3 (<i>Chlorella</i>) 4 (control)]	11 days (Days 4 to 15 of age)	<i>Chlorella</i> sp. [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. [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.	

000026

Algal Flour (*Chlorella*) Notification

Table IV.C-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. regularis</i>						
Rat (Wistar; 6 M/group)	14 days	<i>C. regularis</i> [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> [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
Mouse (CD1; 10 M/group)	10 weeks	<i>C. vulgaris</i> [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> [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 via gavage	Week 1: 0, 125, 250, 500, 1,000, or 2,000 [Doses were doubled during]	<ul style="list-style-type: none"> • Body weight • Food intake • Behavioral changes • Signs of clinical toxicity • Neurological 	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 	Krishnaswamy <i>et al.</i> , 2000

000027

Algal Flour (*Chlorella*) Notification

Table IV.C-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
			the second week to 0, 250, 500, 1,000, 2,000, and 4,000, respectively]	<ul style="list-style-type: none"> examination Clinical chemistry (serum ALT, AST) Urinalysis Organ weights Gross necropsy Histology 	intestines [0, M; 2,000, F] <ul style="list-style-type: none"> 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] Varying grades of chronic interstitial pneumonitis in lungs [≥0] 	
Rat (Sprague-Dawley; 6 M/group)	17 days	<i>C. vulgaris</i> [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> [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]	Lee <i>et al.</i> , 2008

000028

Algal Flour (*Chlorella*) Notification

Table IV.C-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
					↓ 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]	
Rat [CD(SD)IGS; 10 M/group]	10 weeks	<i>C. vulgaris</i> [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> [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> [provided in feed at a concentration of 0 or 30% ⁹]	NR	<ul style="list-style-type: none"> • Growth 	↓ body weight gain and protein efficiency ratios.	Leveille <i>et al.</i> , 1962

000029

Algal Flour (*Chlorella*) Notification

Table IV.C-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 (Holtzman; 10 M/group)	3 weeks	<i>C. pyrenoidosa</i> [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.	
Rat (Harvard; 3 to 4 F/group)	34 days	<i>C. pyrenoidosa</i> [provided in feed at a concentration of 0 or 7%]	0 or 9,249 ⁱ	<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> [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> [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 	↓ 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 ↓ serum LDL-c at 2 weeks [900, 7,200], at 4 weeks [7,200], and at 8 weeks [≥900] ↑ HDL-c at 4 weeks [7,200] ↓ serum TC:HDL-c ratio at 2, 4, or 8 weeks [≥900]	Cherng and Shih, 2005

000030

Algal Flour (*Chlorella*) Notification

Table IV.C-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> [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> [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).

000031

(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*-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* by gavage to pregnant and lactating Swiss albino mice for the first 14 days of gestation and lactation at levels of 0, 100, 300, or 500 mg/kg body weight/day (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* 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* 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 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* 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 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 (Merchant *et al.*, 1990). The symptoms of gastrointestinal upset were noted to generally subside over the rest of the 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) 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

Algal Flour (*Chlorella*) Notification

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* 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 flour (*Chlorella*) (high-lipid or high-protein) under the conditions of intended use.

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

Algal Flour (*Chlorella*) Notification

Table IV.C-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	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	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 [400]. NSD in overall antibody response to influenza	Halperin <i>et al.</i> , 2003

000035

Algal Flour (*Chlorella*) Notification

Table IV.C-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
						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 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
NR, NB, NC	98 subjects (25 to 56 yrs; sex not specified; ulcerative colitis)	2 months	Tablet 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

000036

Algal Flour (*Chlorella*) Notification

Table IV.C-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	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	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
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 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,	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	Merchant and Andre, 2001; Merchant <i>et al.</i> , 2002

000037

Algal Flour (*Chlorella*) Notification

Table IV.C-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
					TG, HDL-c, LDL-c)	vs. baseline and placebo periods. ↓ HDL-c vs. placebo period.	
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 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 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 monocytes, leukocytes, and granulocytes, proportion of lymphocytes bearing specific T-cell and natural killer cell markers)	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: <ul style="list-style-type: none"> • 8/21 subjects (38%) experienced nausea 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> ,	Merchant <i>et al.</i> , 1990

000038

Algal Flour (*Chlorella*) Notification

Table IV.C-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
						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.	
<i>C. regularis</i>							
NR	20 subjects (mean age of 53 yrs; 12 F, 8 M; type II hypercholesterolemia)	3 months	Tablet	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	6.3 g	NE	Adverse events were not reported by the authors.	Lee <i>et al.</i> , 2010

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Algal Flour (*Chlorella*) Notification

Table IV.C-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>Chlorella</i> (strain not specified)							
NR	16 subjects (gender NR; hypercholesterolemia)	3 months	Tablet	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	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) Serum lipids (TC, TG, HDL-c)	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 FBG at 6 months vs. baseline.	Inoue <i>et al.</i> , 1995
<i>Chlorella</i> and <i>Scenedesmus</i> (strains not specified)							
NR	5 healthy males (18 to 23 yrs)	26 days	<i>Chlorella</i> 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	Powell <i>et al.</i> , 1961; Krauss, 1962

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Algal Flour (*Chlorella*) Notification

Table IV.C-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
						<p>were all within normal limits.</p> <p>Adverse events observed included:</p> <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. • Nausea, mild abdominal cramping pain, headache, malaise, and hard bulk stools at level of 500 g algae/day. <p>2 subjects dropped out of the study:</p> <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 	

000041

Algal Flour (*Chlorella*) Notification

Table IV.C-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"> 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; 1 tablet weighs 200 mg and contains >95.5% dried *Chlorella* powder as the active ingredient (Mizoguchi *et al.*, 2008).

000042

IV.F. 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.G. 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.H. Summary and Basis for GRAS

Solazyme intends to market Algal flour (*Chlorella*) (high-lipid and high-protein) produced from the dried biomass of *C. protothecoides* S106 as a food ingredient in a variety of traditional food and beverage products. Algal flour (*Chlorella*) (high-lipid and high-protein) 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 flour (*Chlorella*) (high-lipid and high-protein) were characterized by molecular methods, which showed they were genetically identical. Analytical data support that high-lipid and high-protein Algal flour (*Chlorella*) are consistently manufactured to suitable food grade specifications, including limits set for microbes and heavy metals. Algal flour (*Chlorella*) (high-lipid and high-protein) is stable for up to 148 days under accelerated testing conditions based on the results of an ongoing stability study. A 12-month shelf-life has been designed for Algal flour (*Chlorella*) (high-lipid and high-protein) and formal accelerated stability studies are ongoing.

Algal Flour (*Chlorella*) Notification

Both Algal flour (*Chlorella*) products are intended to be used as ingredients in baked goods and baking mixes, beverages and beverage bases, dairy product analogs, egg products, fats and oils, gravies and sauces, processed vegetables and vegetable juices, and soups and soup mixes. However, an individual food product is intended to contain either the high-lipid or the high-protein product, and not both products at the same time. Thus, the estimated intakes of Algal high-lipid and high-protein flours (*Chlorella*) were calculated as a single Algal flour (*Chlorella*) product. Using data collected in the NHANES 2005-2006 and under the conditions of intended use of Algal flour (*Chlorella*), the total U.S. population mean all-user intake of Algal flour (*Chlorella*) was calculated to be 13.8 g/person/day on an absolute basis or 0.23 g/kg body weight/day on a body weight basis. The heavy consumer (90th percentile) all-user intake of Algal flour (*Chlorella*) from all proposed food-uses by the total population was 29.1 g/person/day, or 0.49 g/kg body weight/day on a body weight basis. Based on the limitations of the methodology used to estimate Algal flour (*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 flour (*Chlorella*) at the maximum specified level of use, it is reasonable to conclude that these intake estimates represent substantial overestimates of the actual Algal flour (*Chlorella*) exposure in the U.S. population that are expected under the proposed uses in food described herein.

The safety of Solazyme's Algal flour (*Chlorella*) (high-lipid and high-protein) under the proposed uses was based on scientific procedures using generally available data. Solazyme's Algal flours (*Chlorella*) are 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 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*.

Algal Flour (*Chlorella*) Notification

Published studies indicate that *Chlorella* species are of low oral toxicity in animals. The NOAEL in a 28-day toxicity study conducted 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). In several studies which evaluated the nutritional value and/or the safety of *Chlorella* species, 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* 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 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 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 flour (*Chlorella*) (high-lipid and high-protein) for use in a food as described in Appendix A at use levels of between 3.5 to 50%,

Algal Flour (*Chlorella*) Notification

is based on the opinion of an Expert Panel of scientists qualified by scientific training and experience to evaluate the safety of Algal flour (*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 flour (*Chlorella*) (high-lipid and high-protein), 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 flour (*Chlorella*) (high-lipid and high-protein), 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 flour (*Chlorella*) (high-lipid and high-protein) 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 flour (*Chlorella*) (high-lipid and high-protein) 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).

VI. References

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Algal Flour (*Chlorella*) Notification

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 sirups	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	173.270	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
	184.1193	Calcium chloride
	184.1212	Calcium pantothenate
	184.1261	Copper sulfate
	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

Algal Flour (*Chlorella*) Notification

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

Algal Flour (*Chlorella*) Notification

Table A-1 Summary of the Individual Proposed Food-Uses and Use-Levels for Algal Flour (<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	30	15
	Bread (excluding sweet quick types) and Rolls	1.5	30	5
	Cakes	9.0	60	15
	Cookies and Brownies	4.5 9.0	30 60	15
	Muffins	9.0	60	15
	Pizza Dough	7.5	50	15
	Quick Breads	4.5	30	15
	Tortillas	4.5	30	15
Beverages and Beverage Bases	Energy Drink Shots ²	3.0	85.13	3.52
Dairy Product Analogs	Low and Reduced Fat Dips (including dry mixes): non-dairy ³	6.4	32	20
	Soy Milk Substitute	12	240	5
Egg Products	Dried Egg Flour	16.5	110	15
Fats and Oils	Low and Reduced Fat-Based Sauces ³	8.4	28	40
	Low and Reduced Fat Margarine and Margarine-Like Spreads	5.6	14	50
	Low and Reduced Fat Mayonnaise and Mayonnaise-Type Dressings	5.6	14	40
	Low and Reduced Fat Salad Dressings (and low calorie, including dry mixes)	5.6	14	40
Gravies and Sauces	Low and Reduced Fat Gravies ³	8.4	28	30
	Low and Reduced Fat Powdered Sauces and Gravies (dry mix)	3.6	12	30
	Tomato-Based Sauces	1.4	28	5
	Low and Reduced Fat White Sauces and Milk Gravies ³	8.4	28	30
Soups and Soup Mixes	Low and Reduced Fat Powdered Soups (cream-based) ³	3.6	12	30
	Prepared Soups (cream-based)	25	245 ⁴	10 20

RTD = Ready-to-drink

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

² No food codes were identified for this category, therefore surrogate codes were used to represent the food codes in this category.

³ No food codes were identified for these categories, thus surrogate codes representing the full-fat versions of these foods were used to represent these categories.

⁴ 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 and Microbiological Batch Analyses
of Algal High-Protein Flour (*Chlorella*) and Algal High-Lipid
Flour (*Chlorella*)**

Algal Flour (*Chlorella*) Notification

Non-consecutive lots of Solazyme's Algal high-protein flour (*Chlorella*) and Algal high-lipid flour (*Chlorella*) were analyzed for chemical and microbiological parameters. The results are presented below in Tables B-1 and B-2.

Table B-1 Summary of the Chemical and Microbiological Product Analysis for 3 Lots of Algal High-Protein Flour (<i>Chlorella</i>)				
Parameter	Specification	Lot Number		
		BMP181	BMP231	BMP310
<i>Proximate</i>				
Moisture (%)	≤10%	4.08	9.0	7.9
Protein (%)	40 to 70%	50.8	51.0	52.2
Fiber (%)	5 to 25%	12.9	14.6	10.31
Ash (%)	<10%	5.98	8.61	7.08
Sucrose (%)	0 to 10%	7.95	7.58	7.71
<i>Lipid Content</i>				
Acid hydrolysis (%)	5 to 25%	15.2	14.23	10.87
Total				
Total Proximate plus Lipid (%)		96.9	105.0	96.9
<i>Heavy Metals</i>				
Arsenic (ppm)	<0.2 ppm	0.004	<0.01	0.0468
Cadmium (ppm)	<0.1 ppm	<0.001	<0.001	0.0269
Chromium (ppm)	<2 ppm	1.15	<0.048	0.211
Lead (ppm)	<0.5 ppm	<0.01	0.07	<0.3
Mercury (ppm)	<0.1 ppm	0.038	<0.005	<0.03
<i>Microbiological</i>				
Aerobic Plate Count (/g)	<10,000 cfu/g	60	100	2,400
<i>E. coli</i>	Negative in 25 g	<10 ¹	Negative	Negative
Salmonella - ELFA (/25g)	Negative in 25 g	Negative	Negative	Negative
Staphylococci	Negative in 25 g	<10 ²	Negative	Negative
Yeast (/g)	<100 cfu/gram	<10	<10	20
Mold (/g)	<100 cfu/gram	30	<10	10

NA = not available; ND = not determined

¹ Lot analyzed using AOAC 966.24 with a limit of <10 cfu/g.

² Lot analyzed using AOAC 975.55 with a limit of <10 cfu/g.

Algal Flour (*Chlorella*) Notification

Table B-2 Summary of the Chemical and Microbiological Product Analysis for 3 Lots of Algal High-Lipid Flour (<i>Chlorella</i>)				
Parameter	Specification	Lot Number		
		BM230	BM147	BM182
Proximate				
Moisture (%)	≤10%	2.84	1.54	4.1
Protein (%)	2 to 15%	4.8	7.3	5.05
Fiber (%)	10 to 50%	29.44	46.6	26.3
Ash (%)	≤10%	2.28	2.49	2.64
Sucrose (%)	0 to 10%	4.78	2.83	4.36
Lipid Content				
Acid hydrolysis (%)	40 to 70%	56.73	42.7	55.85
Total				
Total Proximate plus Lipid (%)		100.9	103.5	98.3
Heavy Metals				
Arsenic (ppm)	<0.2 ppm	<0.01	<0.01	<0.01
Cadmium (ppm)	<0.1 ppm	<0.001	<0.001	<0.001
Chromium (ppm)	<2 ppm	<0.5	<0.5	<0.05
Lead (ppm)	<0.5 ppm	<0.01	0.12	<0.01
Mercury (ppm)	<0.1 ppm	<0.005	<0.005	0.015
Microbiological				
Aerobic Plate Count (/g)	<10,000 cfu/g	<100	<10	<10
<i>E. coli</i>	Negative in 25 g	Negative	<10 ¹	<10 ¹
Salmonella - ELFA (/25g)	Negative in 25 g	Negative	Negative	Negative
Staphylococci	Negative in 25 g	Negative	<10 ²	<10 ²
Yeast (/g)	<100 cfu/g	<10	<10	<10
Mold (/g)	<100 cfu/g	<10	<10	<10

¹ Lot analyzed using AOAC 966.24 with a limit of <10 cfu/g.

² Lot analyzed using AOAC 975.55 with a limit of <10 cfu/g.

**APPENDIX C: Expert Panel Consensus Statement Regarding
the Generally Recognized as Safe (GRAS) Status of *Chlorella*
Powder (High-Fat and High-Protein) for Use in Food**

Expert Panel Consensus Statement Regarding the Generally Recognized as Safe (GRAS) Status of *Chlorella* Powder (High-Fat and High-Protein) 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* powder (high-fat and high-protein) 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* powder (high-fat and high-protein).

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* powder (high-fat and high-protein), 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 POWDER (HIGH-FAT AND HIGH-PROTEIN)

Solazyme's high-fat and high-protein *Chlorella* powder are 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 powder (high-fat and high-protein) is produced 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* powder (high-fat and high-protein) 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. Lipid production is induced by limiting inorganic nitrogen during the latter part of the fermentation process. Following completion of growth, the fermentation broth is harvested, concentrated, optionally washed and/or disrupted, and then dried and packaged. Food-grade antioxidants may be added following the wash stage.

The chemical and microbiological specifications for *Chlorella* high-fat and high-protein powders are presented in Table 1. *Chlorella* high-fat powder is composed of 40 to 70% fat, 2 to 15% protein, and 10 to 40% fiber, with a moisture content of 10% or less. *Chlorella* high-protein powder is composed of 40 to 70% protein, 5 to 25% fat, and 5 to 25% fiber, with a moisture content of 10% or less.

Table 1 Chemical and Microbiological Specifications for <i>Chlorella</i> High-Fat and High-Protein Powder			
Specification Parameter	Specification		Method
	High-Fat	High-Protein	
Proximate			
Moisture Content	≤10%	≤10%	AOAC 930.15
Fiber Content	10 to 50%	5 to 25%	AOAC 991.43
Ash Content	<10%	<10%	AOAC 942.05
Protein Content	2 to 15%	40 to 70%	AOAC 990.03
Fat Content	40 to 70%	5 to 25%	AOAC 954.02
Sucrose	0 to 10%	0 to 10%	AOAC 980.13
Heavy Metals			
Lead	<0.5 ppm	<0.5 ppm	ICP-MS/AOAC 984.27 (microwave) or GLI Procedure ME-30
Arsenic	<0.2 ppm	<0.2 ppm	ICP-MS/AOAC 984.27 (microwave) or GLI Procedure ME-30
Mercury	<0.1 ppm	<0.1 ppm	ICP-MS/AOAC 984.27 (microwave) or GLI Procedure E80-3
Cadmium	<0.1 ppm	<0.1 ppm	ICP-MS/AOAC 984.27 (microwave) or GLI Procedure ME-30
Chromium	<2 ppm	<2 ppm	AOAC 975.03 or AOAC 984.27 or GLI Procedure ME-30
Microbiological Limits			
Aerobic Plate Count	<10,000 cfu/g	<10,000 cfu/g	AOAC 966.23
<i>E. coli</i>	Negative in 25 g	Negative in 25 g	USP31, NF26, 2008
<i>Staphylococci</i>	Negative in 25 g	Negative in 25 g	USP31, NF26, 2008
<i>Salmonella</i>	Negative in 25 g	Negative in 25 g	AOAC 2004.03
Yeast	<100 cfu/g	<100 cfu/g	FDA-BAM, 7 th ed.
Mold	<100 cfu/g	<100 cfu/g	FDA-BAM, 7 th ed.

Analysis of 2 non-consecutive lots of *Chlorella* high-protein powder and 4 non-consecutive lots of *Chlorella* high-fat powder indicate that the manufacturing process produces products that are consistent with their respective specifications, including limits set for heavy metals and microbes.

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* powder (high-fat and high-protein). Formal accelerated stability studies are ongoing.

Both *Chlorella* powder products (high-fat and high-protein) are intended to be used in the same food categories at the same use-levels; however, an individual food product is intended to contain either the high-fat or the high-protein product, and not both products at the same time. Thus, the estimated intakes of *Chlorella* high-fat and high-protein powders were calculated as a single *Chlorella* powder product. Both *Chlorella* powder products are intended to be used as ingredients in baked goods and baking mixes, beverages and beverage bases, dairy product analogs, egg products, fats and oils, gravies and sauces, processed vegetables and vegetable juices, and soups and soup mixes at use-levels ranging from 3.5 to 50%. The complete list of food-uses and use-levels is provided in Appendix 1.

The consumption of *Chlorella* powder 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* powder, the total U.S. population mean all-user intake of *Chlorella* powder was 13.8 g/person/day on an absolute basis or 0.23 g/kg body weight/day on a body weight basis. The heavy consumer (90th percentile) all-user intake of *Chlorella* powder from all proposed food-uses by the total population was 29.1 g/person/day, or 0.49 g/kg body weight/day on a body weight basis.

Although the absorption, distribution, metabolism, and elimination of Solazyme's *Chlorella* powders have not been studied specifically, it is expected that the *Chlorella*-derived proteins, lipids, and carbohydrates will be digested, absorbed, and metabolized through normal physiological processes (PDRNS, 2001), as the macronutrients 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), the compositions of Solazyme's *Chlorella* powders are each 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 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* 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, 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.

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 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 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 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* 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* 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. 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*/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 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* sp., *C. vulgaris*, and *C. pyrenoidosa*, 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* powder (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/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*-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 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* 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 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 (Merchant *et al.*, 1990). The symptoms of gastrointestinal upset were noted to generally subside over the rest of the 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) 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* 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 pheorbide-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 powder (high-fat or high-protein) 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). 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 indicates that the intended uses of *Chlorella* powder (high-fat and high-protein), 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* powder (high-fat and high-protein) 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* powder (high-fat and high-protein) 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* powder (high-fat and high-protein), 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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Professor Joseph F. Borzelleca, Ph.D.
Department of Pharmacology and Toxicology
Virginia Commonwealth University School of Medicine

30 October 2009

Date

(b) (6)

[Redacted signature area]

Professor Eric A. Johnson, Sc.D.
Department of Food Microbiology & Toxicology
University of Wisconsin

10/28/09

Date

(b) (6)

[Redacted signature area]

Professor Gary M. Williams, M.D.
Department of Environmental Pathology & Toxicology
New York Medical College

5 Nov 2009

Date

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Appendix 1

000073

Table A-1 Summary of the Individual Proposed Food-Uses and Use-Levels for <i>Chlorella</i> Powder 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	30	15
	Bread (excluding sweet quick types) and Rolls	1.5	30	5
	Cakes	9.0	60	15
	Cookies and Brownies	4.5 9.0	30 60	15
	Muffins	9.0	60	15
	Pizza Dough	7.5	50	15
	Quick Breads	4.5	30	15
	Tortillas	4.5	30	15
Beverages and Beverage Bases	Energy Drink Shots ²	3.0	85.13	3.52
Dairy Product Analogs	Low and Reduced Fat Dips (including dry mixes): non-dairy ³	6.4	32	20
	Soy Milk Substitute	12	240	5
Egg Products	Dried Egg Powder	16.5	110	15
Fats and Oils	Low and Reduced Fat-Based Sauces ³	8.4	28	40
	Low and Reduced Fat Margarine and Margarine-Like Spreads	5.6	14	50
	Low and Reduced Fat Mayonnaise and Mayonnaise-Type Dressings	5.6	14	40
	Low and Reduced Fat Salad Dressings (and low calorie, including dry mixes)	5.6	14	40
Gravies and Sauces	Low and Reduced Fat Gravies ³	8.4	28	30
	Low and Reduced Fat Powdered Sauces and Gravies (dry mix)	3.6	12	30
	Tomato-Based Sauces	1.4	28	5
	Low and Reduced Fat White Sauces and Milk Gravies ³	8.4	28	30
Soups and Soup Mixes	Low and Reduced Fat Powdered Soups (cream-based) ³	3.6	12	30
	Prepared Soups (cream-based)	25	245 ⁴	10.20

RTD = Ready-to-drink

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

² No food codes were identified for this category, therefore surrogate codes were used to represent the food codes in this category.

³ No food codes were identified for these categories, thus surrogate codes representing the full-fat versions of these foods were used to represent these categories.

⁴ Serving sizes based on Reference Amounts Customarily Consumed (RACC) in the U.S. CFR (21 CFR § 101.12) (U.S. FDA, 2009).

Submission End

000075

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