Request for an Opinion on the Substantial Equivalence of an Astaxanthin-Rich Extract of *Haematococcus pluvialis* algae (BioAstin®) with the Existing Whole-Algal Product (Astaxin™/AstaCarox™), for Use in Human Dietary Supplements

Submitted to the UK Food Standards Agency Under Article 5 of EC Regulation 258/97, by Cyanotech Corporation, Kailua-Kona, Hawaii, USA

Introduction

Cyanotech Corporation intends to market its natural astaxanthin product, BioAstin®, in the EU for use in dietary supplements. BioAstin® is an oleoresin of the common green algae *Haematococcus pluvialis*, extracted from the dried algal biomass with supercritical carbon dioxide (CO_2). We intend to make a notification in accordance with Article 5 of Regulation (EC) No 258/97 regarding novel foods and novel food ingredients, utilizing the simplified procedure for algae-based products that can be shown to be substantially equivalent to existing foods. This procedure requires a suitable opinion from a competent authority in one of the EU member states. Accordingly, we are directing this request for a substantial equivalency opinion to the UK competent authority (Food Standards Agency).

In this application, we present our case that our extracted *H. pluvialis* product, BioAstin®, is substantially equivalent to the existing whole-algal product produced by the Swedish company AstaCarotene AB. AstaCarotene, now owned by Fuji Chemical Industry Co., Ltd. of Japan and renamed AstaReal AB, has marketed its Astaxin™ product in the EU since at least 1995. Astaxin™ is a dietary supplement consisting of hard gelatin capsules containing the dried biomass of *H. pluvialis*. In bulk form, this whole algal product is marketed to other supplement manufacturers under the trade name AstaCarox™. As required by Article 3(4) of Regulation (EC) No 258/97, we present comparative data and information on these products regarding the five stated criteria: composition, nutritional value, metabolism, intended use and level of undesirable substances.

Haematococcus pluvialis algae are naturally rich in carotenoids, principally astaxanthin. The algae have been used in human dietary supplements for over a decade and have an established record of safety. BioAstin® is a lipid extract of *H. pluvialis* algae, and therefore represents a subset of the components that may be found within the algae biomass. BioAstin® is available at two standardized astaxanthin titers: 10% (BioAstin® SCE10) and 5% (BioAstin® SCE5). A similar 10% astaxanthin CO_2-extracted *H. pluvialis* product, Zanthin®, is produced by the US company Valensa, Inc. (formerly US Nutra LLC). In 2004, the Food Standards Agency confirmed the substantial equivalency of Zanthin® with AstaCarotene's existing whole algal product. We present data and information comparing BioAstin® with Zanthin® extract, and demonstrate that their compositions and production processes are highly similar.

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1. Administrative Information

1.1. Applicant

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1.2. Name of Novel Food Ingredient

BioAstin® Natural Astaxanthin, a CO_2-extracted oleoresin of *Haematococcus pluvialis* microalgae.

1.3. Date of Application

17 May 2006

2. Composition

2.1. Specifications

2.1.1. General Specifications

Table 2.1.1.1. General specifications of BioAstin® Natural Astaxanthin

Component	Specification	
	BioAstin® SCE5	BioAstin® SCE10
protein	<3%	<5%
carbohydrates	<30%	<55%
fat	>60%	>40%
dietary fiber	<1%	<1%
ash	<2%	<2%
moisture	<8%	<8%
total astaxanthin	>5%	>10%

2.1.2. Source Organism

BioAstin® Natural Astaxanthin is a CO_2-extracted oleoresin of the common green algae *Haematococcus pluvialis*. This microalgal species is classified as follows (Melkonian 1990):

Phylum: Chlorophyta
Class: Chlorophyceae
Order: Chlamydomonadales
Family: Haematococcaceae
Genus: *Haematococcus*
Species: *pluvialis*

Haematococcus pluvialis, also referred to as *Haematococcus lacustris* or *Sphaerella lacustris* in the older literature, is a ubiquitous green alga. Hazen (1899) reported that the alga "is reported as very common and widely distributed in Europe, where it is found from Scandinavia to Venice...the alga is distributed from Vermont to Texas and from Massachusetts to Nebraska and probably farther West." Its typical habitat as ephemeral rock pools and other temporary bodies of water.

Haematococcus algae cells can be readily identified in culture by their size, motility, number of flagella (two), patterns of division and especially, by their formation of blood-red resting cysts under exposure to environmental stress. Dried, ruptured *Haematococcus* cysts may be further identified by spectrophotometric analysis of astaxanthin following its extraction into an organic

solvent. The astaxanthin produced by *H. pluvialis* can also be differentiated from astaxanthin derived from other sources by thin-layer chromatography or by high-pressure liquid chromatography.

The algal strain cultivated at Cyanotech for production of *Haematococcus* algae meal is *Haematococcus pluvialis* Flotow strain Steptoe. The source culture was isolated in the Steptoe watershed of Nevada, USA, by Dr. Ralph Lewin of the Scripps Institution of Oceanography. This strain has been maintained in pure laboratory culture since its isolation, and has undergone no genetic manipulation. This algal strain, which we refer to as H2B, is identical to that described in Cifuentes et al. (2003). Because the production strain has never been genetically modified, it is considered non-GM. Although the exact strain of *H. pluvialis* used by AstaCarotene is a trade secret, the known *H. pluvialis* strains are expected to be phytochemically similar if cultivated in a similar manner (Wilkinson 2004). The compositional data provided in this dossier confirms this similarity.

2.1.3. Production Methods

Cultivation and initial processing of the *Haematococcus* microalgae used for BioAstin® Natural Astaxanthin are carried out by Cyanotech Corporation in Hawaii, USA. The location of Cyanotech's production facility is:

Cyanotech Corporation
73-4460 Queen Kaahumanu Highway, #102
Kailua-Kona, HI 96740
Telephone: 808-326-1353
FAX: 808-329-3597

Approximately 35% of Cyanotech's 90 acre facility is devoted to *Haematococcus* algae production. This area includes 25 production ponds and their associated inoculation and scaleup systems, 2 laboratories for analysis, and all equipment necessary for the processing of *Haematococcus* algae biomass including mills, spray dryers, and packaging equipment. Cyanotech Corporation employs 65 associates, 10 of whom are dedicated to the manufacture of *Haematococcus* algae meal.

The steps in the manufacturing of BioAstin® are confidential and are presented in CONFIDENTIAL Appendix 1 2006. However the general manufacturing process for *Haematococcus* algae meal has been outlined in Lorenz and Cysewski (2000). This process is also similar to those utilized by other *Haematococcus* astaxanthin manufacturers, as discussed by Olaizola and Huntley (2003).

2.1.3.1. Extraction and Standardization

The extraction process utilizes supercritical carbon dioxide (CO_2) to remove the lipid fraction, including carotenoids, from the dried Haematococcus algae. The process is similar to that used extensively in the food industry to defat a variety of nuts and other products and utilizes cGMP procedures for all processing. The extracted product (BioAstin®) is a thick liquid oleoresin composed of ~8-12% astaxanthin. The identity of the extraction company and details of the extraction procedure may be found in a CONFIDENTIAL Appendix 2 2006. The extraction procedure uses pure CO_2 as a solvent, with no cosolvents or entrainers. This extraction procedure is quite similar to the one used by US Nutra for the production of Zanthin® extract (US Nutra 2004d).

The raw extract is shipped back to Cyanotech in sanitary polyethylene drums for standardization. Lots of raw extract are blended in a stainless steel mixing vat to produce a product with guaranteed minimum 10% astaxanthin (BioAstin® SCE10). To produce a 5% astaxanthin product (BioAstin® SCE5), non-GM high-oleic safflower oil (Oilseeds2004) is blended with the raw extract.

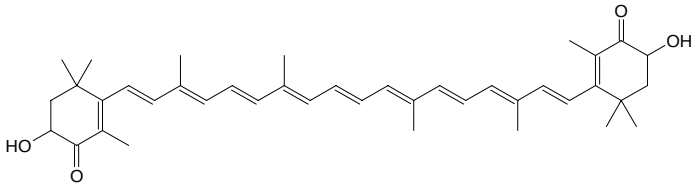
2.1.4. General Composition

Table 2.1.4.1. General composition of BioAstin® Natural Astaxanthin and *Haematococcus pluvialis* algae biomass

Component	Percent of Total Weight (mean ±sd)		
	BioAstin® SCE5*	BioAstin® SCE10†	*Haematococcus* Algae Meal‡
protein	1.3±0.2	3.3±1.1	28.6±1.3
carbohydrates	26.4±4.0	47.3±5.5	44.8±3.3
fat	67.2±7.8	42.8±0.6	13.1±0.1
dietary fiber	BQL**	BQL	19.2±3.2
ash	BQL	1.2±1.4	6.7±1.1
moisture	5.1±5.5	5.5±2.5	6.8±1.0
total astaxanthin§	5.28±0.13	10.57±0.43	2.58±0.10

*Proximate data based on analyses of 3 lots (Covance2004d, Covance2005a, Covance 2005b)
†Proximate data based on analyses of 2 lots (Covance2002a, Covance2002b)
‡Proximate data based on analyses of 3 lots (Covance2004a, Covance2004b, Covance2004c)
§HPLC results are reported in Cyanotech Corporation 2006a, Cyanotech Corporation 2006b and Cyanotech Corporation 2004d
**BQL = below quantification limit

2.1.5. Properties of Astaxanthin

Table 2.1.5.1. Physical and chemical properties of astaxanthin	
Property	Value
generic name	astaxanthin
formal (IUPAC) name	3,3'-dihydroxy-β,β-carotene-4,4'-dione
CAS Registry No.	472-61-7
molecular formula	$C_{40}H_{52}O_4$
molecular weight	596.82
structural formula (all-E isomer)	
melting point	182-183 °C
solubility in water	insoluble
solubility in chloroform	5 mg/ml
solubility in DMSO	50 mg/ml
visible absorption spectra	see Buchwald and Jencks (1968)
geometrical isomers	272 possible; only 4 common (all-E, 9Z, 13Z, 15Z)
stereoisomers	3 possible: (3S,3'S), (3R,3'S), (3R,3'R)

The astaxanthin molecule has a number of geometrical (E/Z) isomers and stereoisomers (R/S). These are described in detail and their structures presented in Østerlie et al. (1999). In *Haematococcus* algae, the all-E form of astaxanthin is predominant, while smaller amounts of 9Z, 13Z, 15Z and diZ astaxanthin are also present. *Haematococcus* algae contain optically pure (3S,3'S)-astaxanthin (Renstrøm et al. 1981; Grung et al. 1992), the same stereoisomer predominately accumulated by wild salmonid fish (Bjerkeng 1997). The astaxanthin found in *Haematococcus* algae is mainly esterified with fatty acids. Monoesters are most abundant, followed by diesters and free astaxanthin (Renstrøm et al. 1981).

2.1.6. Assay Methods

2.1.6.1. General Composition and Contaminant Levels

The proximate composition, fatty acid composition and microbiological, heavy metal and pesticide contaminant levels in BioAstin® are analyzed by Covance Laboratories, Inc., in Madison, Wisconsin, USA. The standard methods used by

Covance are described in the individual analysis reports (Covance2004d, Covance2005a, Covance2005b, Covance2002a, Covance2002b).

2.1.6.2. Carotenoids

The qualitative composition of the principal carotenoids in the additive may be determined by thin-layer chromatography (TLC). Using the solvent system described by Grung et al. (1992), the major carotenoids of Cyanotech's *Haematococcus* algae meal have been identified and compared with those of krill oil, synthetic astaxanthin and *Phaffia rhodozyma* yeast (Cyanotech Corporation 2004b). The predominance of esterified astaxanthin in *Haematococcus* algae meal and krill oil clearly distinguish them from the other two astaxanthin sources.

A rough approximation of the astaxanthin content of *Haematococcus* algae meal may be obtained using spectrophotometry (Boussiba et al. 1992), however, high-pressure liquid chromatography (HPLC) is preferred, because it allows for separation of astaxanthin from other pigments. Although there are a number of HPLC methods commonly used for the analysis of free astaxanthin, the quantitative analysis of esterified astaxanthin is more problematic, because of the dearth of authentic standards for these astaxanthin derivatives. Anaerobic saponification may be used to convert astaxanthin esters to free astaxanthin (Renstrøm et al. 1981; Grung et al. 1992), but in practice, complete recovery of the astaxanthin is difficult to achieve and significant amounts of unwanted reaction products may be created.

The quantitative analysis of astaxanthin in *Haematococcus* algae meal and BioAstin® extract are carried out according to the methods described in Cyanotech Corporation (2003a) and Cyanotech Corporation (2003b), respectively. In these methods, astaxanthin esters are cleaved prior to HPLC analysis, using the enzyme cholesterol esterase. This enzymatic de-esterification has been shown to be facile and to produce high yields of the free carotenoid and low yields of undesired reaction products (Jacobs et al. 1982). Once the astaxanthin is all in the free form, it is separated and quantified by HPLC, following protocols slightly modified from Vecchi et al. (1987) and Schüep and Schierle (1995). The Cyanotech method has been reviewed and accepted by the U.S. FDA in conjunction with 21 CFR part 73.185, regarding the use of *Haematococcus* algae meal as a color additive in salmonid feeds. Additionally it has been accepted by the Canadian Food Inspection Agency as a method to quantify astaxanthin in salmonid feeds within Feed Additive Petition CFIA 990535. The Cyanotech method and variations thereof are also used regularly by the Japan Food Research Laboratory to analyze a variety of *Haematococcus* products.

2.1.7. Maximum Limits for Contaminants

BioAstin® Natural Astaxanthin (both SCE5 and SCE10) adheres to strict limits for levels of microbiological and heavy metal contaminants. These specifications are listed in the tables below. BioAstin® is also guaranteed to be free from chemical pesticides (organophosphates, organonitrogens and organochlorinated compounds).

Table 2.1.7.1. Microbiological specifications of BioAstin® Natural Astaxanthin

Microbiological Contaminant	Specification (cfu/gram)
total aerobic bacteria	$<10^3$
total coliforms	<10
E. coli	negative
yeasts and molds	$<10^2$
Salmonella	negative
Staphylococcus aureus	negative

Table 2.1.7.2. Heavy metal specifications of BioAstin® Natural Astaxanthin

Heavy Metal Contaminant	Specification (ppm)
arsenic	<2
cadmium	<0.1
lead	<2
mercury	<0.025

2.2. Comparison with Existing Food Ingredient

2.2.1. Extracted Oleoresin vs. Whole Algae

The Swedish company AstaCarotene AB (now AstaReal AB, a subsidiary of Fuji Chemical Industry Co., Ltd.) has been marketing its Astaxin™ natural astaxanthin product in the EU since at least 1995. This dietary supplement consists of hard gelatin capsules containing the dried biomass of *Haematococcus pluvialis* algae. AstaCarotene (AstaReal/Fuji) also markets the algal biomass in bulk to other supplement manufacturers under the trade name AstaCarox™.

This application concerns the product BioAstin®, produced by the US company Cyanotech Corporation. BioAstin® is an oleoresin of *H. pluvialis*, extracted from the algal biomass using supercritical carbon dioxide (CO_2). BioAstin® is available at two standardized astaxanthin titers: 10% (BioAstin® SCE10) and 5%

(BioAstin® SCE5). Non-GM high-oleic safflower oil is used to dilute the oleoresin when making the SCE5 product.

BioAstin® SCE10 is nearly identical in composition to Zanthin®, a 10% astaxanthin CO_2-extracted *H. pluvialis* product manufactured by US Nutra LLC (now called Valensa, Inc.) in the US. The algae meal used for production of Zanthin® is cultivated and processed in Israel by Algatechnologies, Ltd. Zanthin® was previously determined by the UK Food Standards Agency to be substantially equivalent (AvdCommNovFood2004) to AstaCarotene's existing whole algal product, based primarily upon the recognition that the former is essentially a subset of the constituents of the latter. The analytical results tabulated below demonstrate the similarity between BioAstin®, Zanthin®, their source algae biomass and the existing whole algal product (Astaxin™/AstaCarox™).

2.2.2. Fatty Acid Profiles

The fatty acid compositions of all the algae biomass and extracted oleoresin products are quite similar, and are dominated by 16:0, 18:1, 18:2 and 18:3 acids. The relatively high and variable 18:1 (oleic acid) content of BioAstin® SCE5 is due to the use of high-oleic safflower oil as a diluent for astaxanthin standardization. Additional small differences in the levels of specific fatty acids may be explained by some variability between *H. pluvialis* strains and/or some analytical variability between the laboratories conducting the analyses. Also potentially contributing to these small differences is the extent to which unsaturated fatty acids have been converted to saturated fatty acids within the algal cells during the astaxanthin accumulation step of cultivation (Zhekisheva et al. 2002).

Table 2.2.2.1. Comparative fatty acid compositions of *Haematococcus* algae meals and extracts						
Fatty Acid	Percent of Total Fatty Acids (mean±sd)					
	Haematococcus algae meals			*Haematococcus* algae extracts		
	Cyanotech*	AstaCarox™†	Alga-technologies‡	BioAstin® SCE5§	BioAstin® SCE10**	Zanthin®††
14:0	0.6±0.02	0.4	0.4±0.01	0.3±0.04	0.4±0.07	0.5%
15:0	0.2±0.02	ND‡‡	0.4±0.02	0.2±0.11	BQL§§	0.0%
16:0	24.7±0.16	17.6	11.8±0.06	12.0±0.97	19.6±0.38	12.2%
16:1	1.0±0.13	ND	0.3±0.01	0.5±0.09	1.0±0.16	0.3%
17:0	0.2±0.0	ND	2.1±0.02	0.1±0.04	0.2±0.00	0.1%
17:1	0.0	3.5	5.4±0.03	0.7±0.92	1.5±0.44	1.9%
18:0	1.2±0.13	3.5	0.6±0.01	1.5±0.43	1.9±1.02	0.8%
18:1	27.5±0.16	31.3	21.1±0.06	44.1±16.51	24.9±2.30	24.1%
18:2 n-6	29.5±1.08	28.5	30.5±0.05	20.0±0.17	32.8±4.60	30.7%
18:3 n-6	2.3±0.12	1.3	14.4±0.02	0.9±0.25	1.4±0.59	14.8%
18:3 n-3	BQL	11.6	0.1±0.01	12.1±3.63	14.3±1.79	0.2%
20:0	0.4±0.01	0.6	2.0±0.02	0.5±0.22	0.5±0.03	1.8%
20:1	0.0±0.02	0.2	0.2±0.01	0.4±0.08	0.3±0.00	0.3%
20:2 n-6	0.5±0.03	0.1	0.5±0.01	0.2±0.11	0.3±0.00	0.5%
20:3 n-6	0.1±0.02	ND	1.3±0.02	0.1±0.00	BQL	1.3%
20:4 n-6	1.1±0.03	0.8	6.8±0.10	0.5±0.18	0.6±0.13	0.1%
20:5 n-3	ND	0.2	ND	ND	ND	0.1%
21:0	ND	ND	1.1±0.02	ND	ND	1.7%
22:0	0.3±0.01	0.2	ND	0.3±0.03	0.2±0.00	0.1%
22:1	ND	ND	0.2±0.01	ND	ND	0.1%
22:6	ND	0.1	ND	ND	ND	ND

*Based on analyses of 3 lots (Covance2004a, Covance2004b, Covance2004c)
†Average results (no sd given) from 3 lots (Fuji 2005)
‡Based on analyses of 2 lots (US Nutra 2004a)
§Based on analyses of 3 lots (Covance2004d, Covance2005a, Covance2005b)
**Based on analyses of 2 lots (Covance2002a, Covance2002b)
††Based on analyses of 1 lot (US Nutra 2004b)
‡‡ND = no data
§§BQL = below quantification limit

Figure 2.2.2.1. Fatty acid compositions of *Haematococcus* algae meals

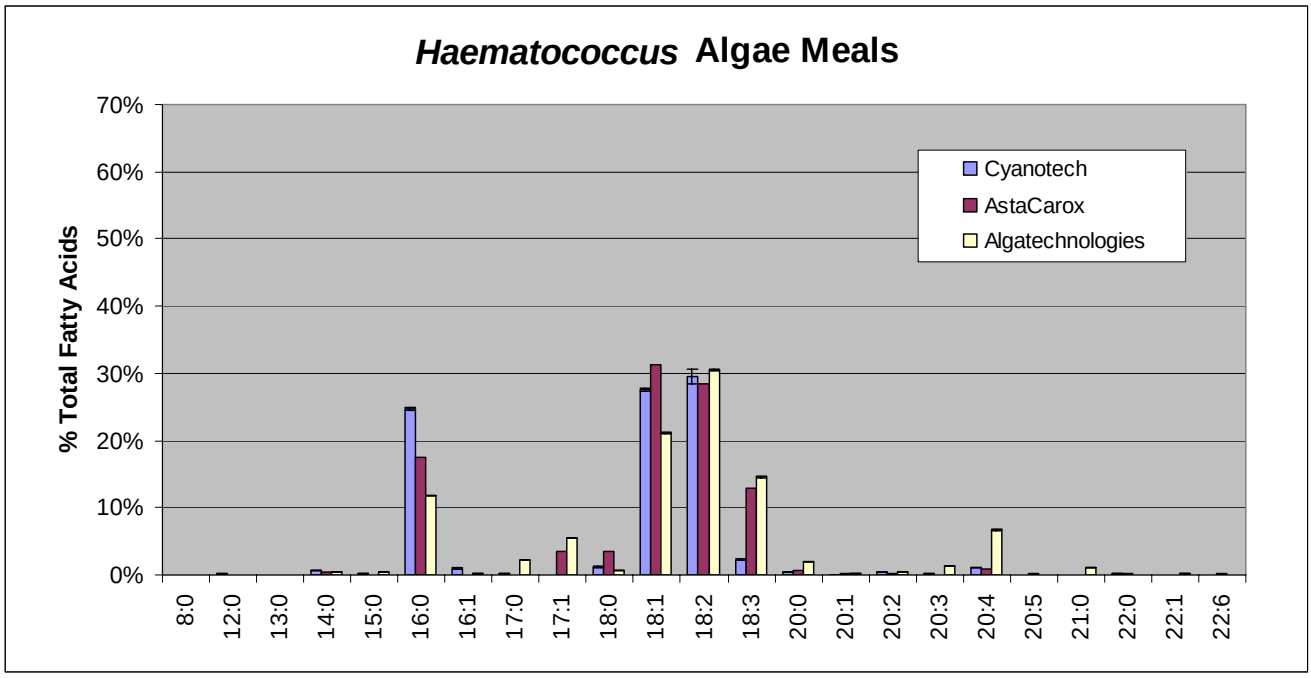
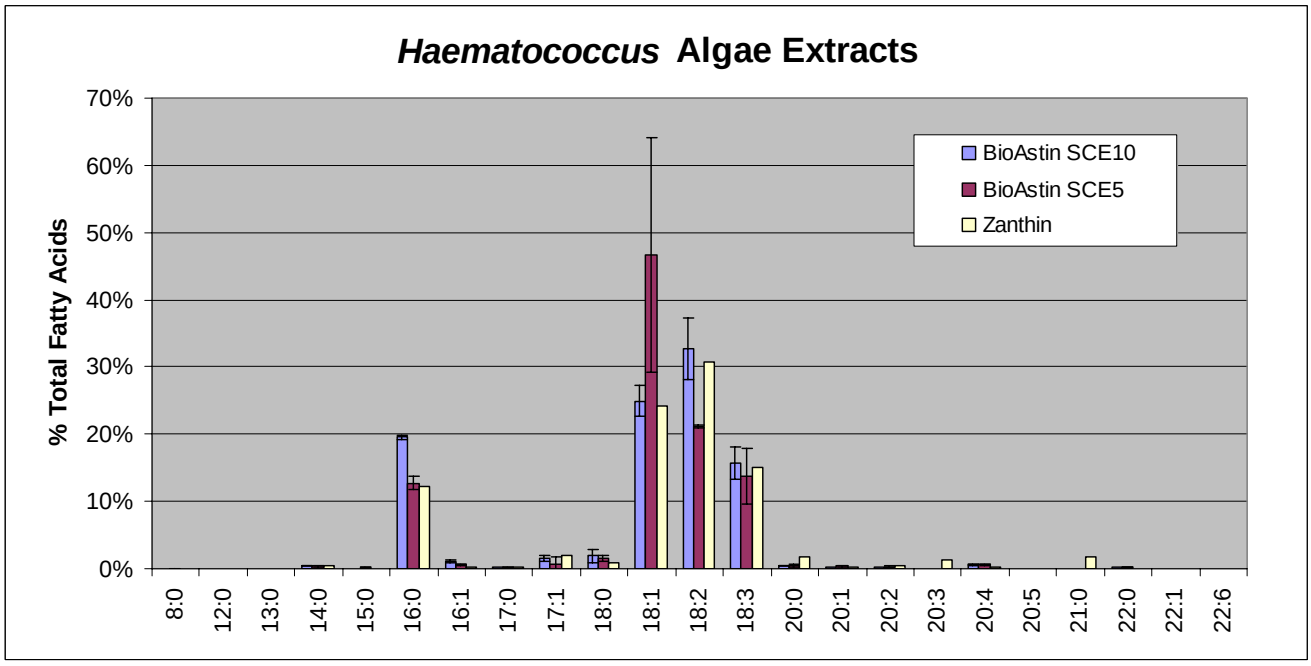


Figure 2.2.2.2. Fatty acid compositions of *Haematococcus* algae extracts



2.2.3. Carotenoid Profiles

The carotenoid profiles of all the algae biomass and extracted oleoresin products are quite similar. Total astaxanthin represents some 2-4% of the total weight of the algae meals, while canthaxanthin, β-carotene and lutein make small contributions. No isomeric data is available for AstaCarox™, but the algae meals

produced by Cyanotech and Algatechnologies have all-E astaxanthin as the dominant geometrical isomer, with lesser but quantifiable contributions by the 9Z and 13Z isomers. A similar pattern is observed in the carotenoid profiles of the extracted products BioAstin® SCE10 and Zanthin®, with an approximate concentration factor of 3-4 from the source algal meal. The astaxanthin in BioAstin® SCE5 is diluted by a factor of approximately 2 from that of the BioAstin® SCE10, but still displays the same relative proportions of geometrical isomers.

Table 2.2.3.1. Comparative carotenoid compositions of *Haematococcus* algae meals and extracts

Carotenoid	Percent of Total Weight (mean±sd)					
	Haematococcus algae meals			*Haematococcus* algae extracts		
	Cyanotech*	AstaCarox™†	Alga-technologies‡	BioAstin® SCE5§	BioAstin® SCE10**	Zanthin®††
astaxanthin all-E	1.94±0.13	ND‡‡	2.60±0.09	3.71±0.02	7.02±0.60	5.92
astaxanthin 9Z	0.35±0.02	ND	0.07±0.01	1.00±0.13	1.95±0.44	1.48
astaxanthin 13Z	0.25±0.05	ND	0.49±0.09	0.46±0.03	1.22±0.57	2.58
astaxanthin 15Z	0.02±0.01	ND	ND	0.04±0.01	0.06	NR
astaxanthin diZ	0.03±0.01	ND	ND	0.07±0.02	0.19±0.14	NR
total astaxanthin	2.58±0.10	3.80	3.16±0.12	5.28±0.13	10.47±0.61	9.98
β-carotene	0.06±0.01	0.02	0.14±0.00	0.13±0.02	0.26±0.07	0.03
canthaxanthin	0.05±0.01	0.02	0.01±0.00	0.14±0.04	0.15±0.05	0.03
lutein	0.13±0.02	0.02	0.04±0.00	0.22±0.02	0.18±0.10	0.07

*Based on analyses of 3 lots (Cyanotech Corporation 2004d)
†Average results (no sd given) from 3 lots (Fuji 2005)
‡Based on analyses of 2 lots (US Nutra 2004a)
§Based on analyses of 3 lots (Cyanotech Corporation 2006b)
**Based on analyses of 4 lots (Cyanotech Corporation 2006a)
††Based on analyses of 1 lot (US Nutra 2004b)
‡‡ND = no data

Figure 2.2.3.1. Carotenoid profiles of *Haematococcus* algae meals

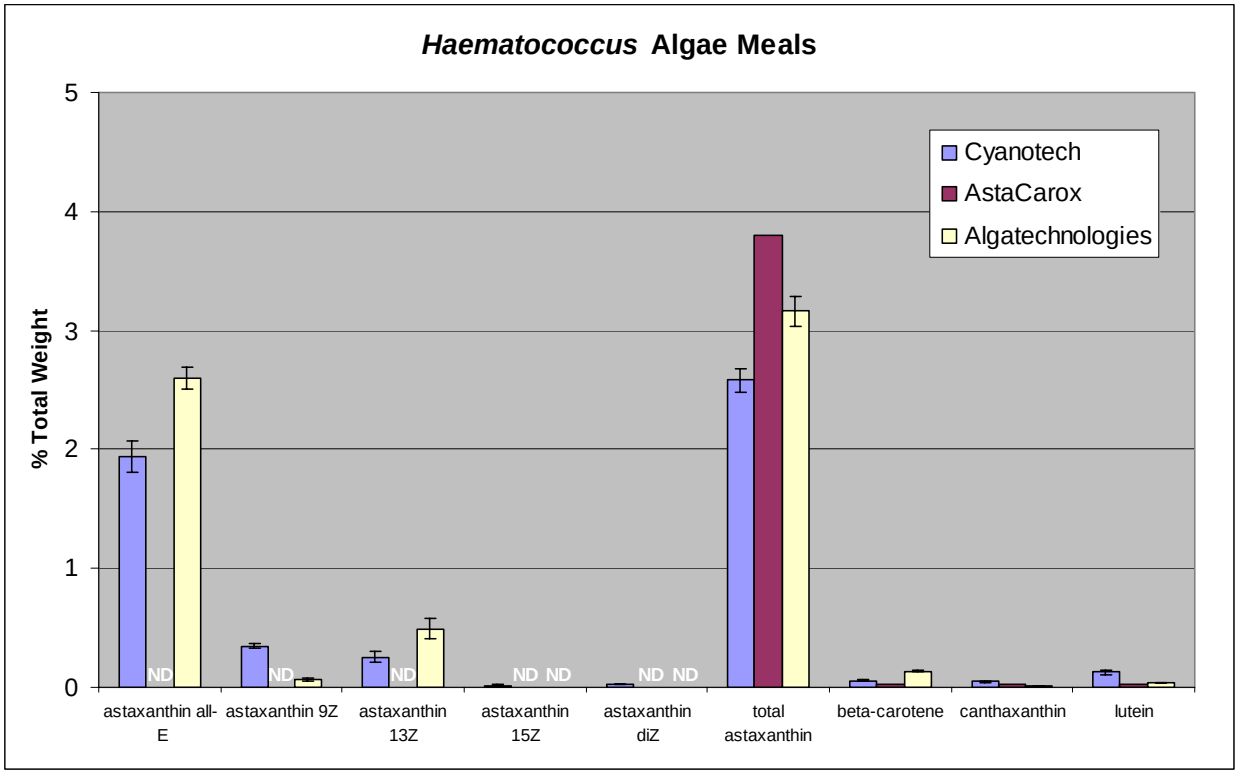
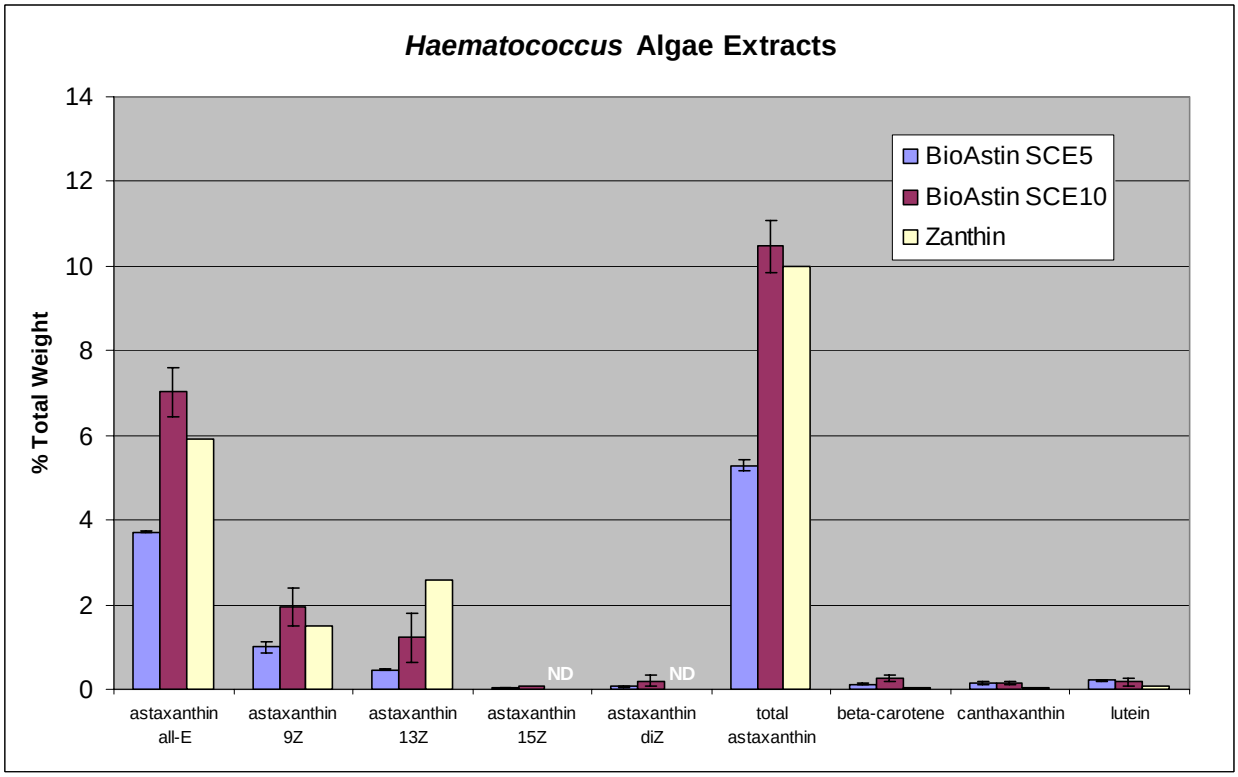


Figure 2.2.3.2. Carotenoid profiles of *Haematococcus* algae extracts

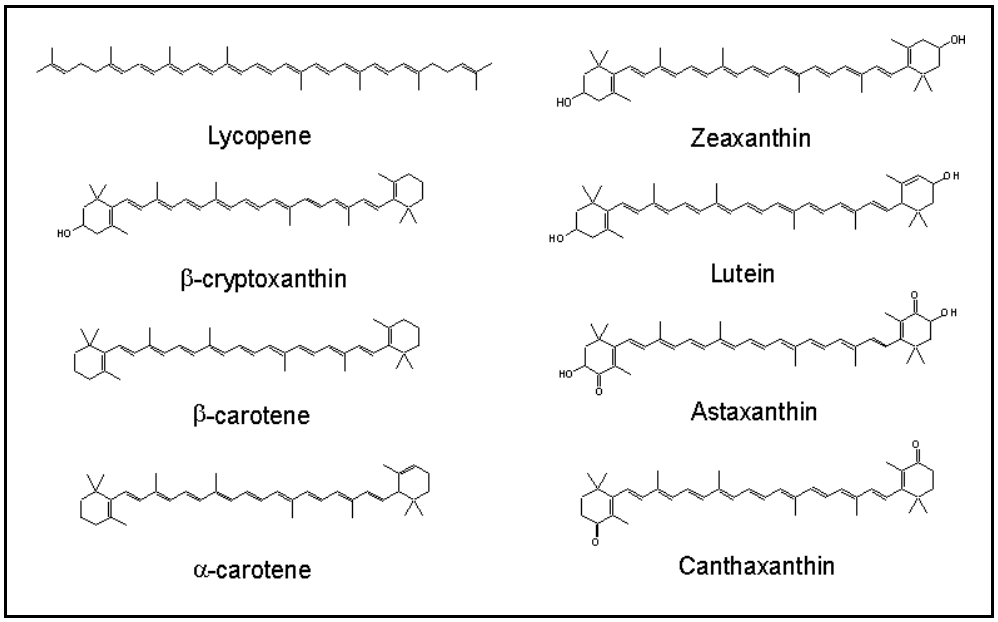


3. Nutritional Value

3.1. Dietary Carotenoids

Antioxidant-rich foods in the diet are believed to contribute to the long-term health of all mammals. Carotenoids are important biological antioxidants that naturally occur in a wide variety of foods. These compounds are thought to support immune function and help to reduce cellular DNA damage associated with aging. The nutritional benefits of carotenoids extend beyond the role of some carotenoids as precursors of vitamin A. For example, the xanthophyll (oxygenated) carotenoids lutein and astaxanthin, and the carotene carotenoid lycopene, have all exhibited potential health benefits to animals and humans in scientific studies, yet none of them is a source for vitamin A (Rao and Agarawal 2000, Shao 2001, Guerin et al. 2003).

Figure 3.1.1. Structures of selected carotenoids



3.2. Astaxanthin

Astaxanthin, a xanthophyll (oxygenated) carotenoid, is known to be among the most potent antioxidant carotenoids in vitro and in vivo, yet unlike β-carotene it does not display any pro-oxidant behavior, even at high concentration and high oxygen tension (Dore 2005). Astaxanthin is at least an occasional component in the human diet due to its presence in fish and crustaceans. Among human populations that consume large amounts of salmon, dietary astaxanthin may be more significant.

Haematococcus algae have a naturally high astaxanthin content. As a source of astaxanthin, the algal product is therefore practical for addition to dietary

supplements than alternative sources such as salmon and shrimp meals, which may contain only a few ppm or less of astaxanthin. Lipid extracts of *Haematococcus* (such as BioAstin®), with astaxanthin concentrations from 5-10%, are even more practical for this purpose. BioAstin® Natural Astaxanthin is not a drug – it is not intended to prevent, cure, treat or mitigate any disease or specific condition. BioAstin® is also not a vitamin; unlike β-carotene, astaxanthin has no provitamin A activity.

Astaxanthin has long been used as a pigmenting agent in diets for aquacultured salmon and trout. Although the purpose of added astaxanthin in feeds for salmonid fishes is to impart coloration to fish flesh, it has recently been suggested that carry-over of astaxanthin from farmed salmon into the human food chain should be beneficial to human health (Baker and Günther 2004). This viewpoint is based upon both the longstanding recognition of wild salmon as a healthful food and the growing body of scientific evidence revealing positive health effects of dietary astaxanthin on human beings and rodent models. These apparent effects include improvement of joint health, protection from sunburn, prevention of age-related macular degeneration, prevention of some types of cancer, enhancement of the immune system, and many others. At least six review articles concerning the potential use of astaxanthin in human health management have been published since 2000, and may be consulted for further details and references on this subject (Maher 2000; Naguib 2001; Cronin 2002; Guerin et al. 2003; Wiener et al. 2003; Dore 2005).

4. Metabolism

4.1. Animal Models

4.1.1. Fishes

The metabolism of astaxanthin in salmonid fishes has been intensively studied due to its commercial importance as a pigmenting agent in farmed trout and salmon. Detailed reviews have been published (e.g., Torrissen et al. 1989; Bjerkeng 2000; FEEDAP 2005).

4.1.2. Rodents

Astaxanthin metabolism was investigated in primary cultures of rat hepatocytes, and was found to proceed via an asymmetric cleavage at the 9,9' positions, consistent with its lack of provitamin A activity in mammals (Wolz et al. 1999). Two metabolites were identified: (rac)-3-hydroxy-4-oxo-β-ionone and its reduced form, (rac)-3-hydroxy-4-oxo-7,8-dihydro-β-ionone. This metabolic pathway, quite different from that occurring in fish, was later found to be in common with that occurring in primary human hepatocytes and human volunteers (Kistler et al. 2002).

In mice, orally administered free astaxanthin has been found to rapidly appear in the plasma and liver (Kurihara et al. 2002). Esterified astaxanthin, administered either as a synthetically produced diester (Showalter et al. 2004) or as an extract of *Haematococcus* algae (Aoi et al. 2003), also is quickly assimilated and appears as free astaxanthin in the serum, liver, heart, gastrocnemius muscle, and to a lesser extent, the brain. The patterns of accumulation for free and esterified astaxanthin in mice are different: free astaxanthin appears more rapidly in the liver than in the serum when the source astaxanthin is esterified (Showalter et al. 2004). This finding suggests a greater bioavailability in mice of esterified astaxanthin, such as that from *Haematococcus*, than free astaxanthin.

4.1.3. Dogs and Cats

Three studies of astaxanthin supplementation in companion animals were presented at the 2004 Experimental Biology Meeting in Washington, DC, and their abstracts were published in Volume 18 of the FASEB Journal. Although the complete studies have not yet been published, the experimental details and associated figures appear in the United States Patent Application No. US 2004/0151761 A1 (Chew et al. 2004a). In each of these studies, the source of the astaxanthin used for supplementation was an oleoresin extracted with supercritical CO_2 from the dried biomass of *Haematococcus* algae. In the first study (Chew et al. 2004b), female Beagle dogs were supplemented orally with up to 40 mg of *Haematococcus* astaxanthin daily for 16 days. Female domestic short hair cats were supplemented orally with up to 10 mg of *Haematococcus* astaxanthin daily for 15 days. Dietary astaxanthin was absorbed into the plasma of both dogs and cats in a time and dose-dependent fashion. Astaxanthin was transported mainly by HDL and was incorporated into all subcellular organelles of blood leukocytes. In the second study, (Chew et al. 2004c), female Beagle dogs were supplemented orally with up to 40 mg of *Haematococcus* astaxanthin for 16 weeks. Astaxanthin was absorbed into the plasma in a dose-dependent fashion. In the third study (Chew et al. 2004d), female domestic short hair cats were supplemented orally with up to 10 mg of *Haematococcus* astaxanthin daily for 12 weeks. Astaxanthin was absorbed into the plasma in a dose-dependent fashion. There were no differences in body weight or blood hematology between supplemented and control animals noted in any of these three studies.

4.2. Humans

The appearance, pharmacokinetics and distribution of astaxanthin geometrical and stereoisomers in plasma and lipoprotein fractions were studied in human volunteers after ingestion of a meal containing 100 mg of synthetic free astaxanthin (Østerlie et al. 2000). The maximum plasma astaxanthin concentration of 1.3±0.1 mg l-1 was reached in 6.7±1.2 hours, and the plasma elimination half-life was 21±11 hours. 13Z-astaxanthin accumulated selectively, while the stereoisomeric composition was unchanged. Astaxanthin was present in

all lipoprotein fractions but was found mainly in very low-density lipoproteins containing chylomicrons. Similar results were found when human volunteers were given fatty acyl diesters of astaxanthin rather than free astaxanthin (Coral-Hinostroza et al. 2004), except that the pharmacokinetics was slower (peak plasma astaxanthin concentration reached at 11.5 hours, plasma elimination half-life = 52±40 hours). No astaxanthin esters were found in the plasma, suggesting that hydrolysis of the esters before uptake may be the rate-limiting step in bioabsorption. However, the oral bioavailability of esterified astaxanthin administered as *Haematococcus* algae meal has been shown to be enhanced in human volunteers by the incorporation of the astaxanthin into lipid-based formulations, suggesting that dissolution from the matrix and/or incorporation into mixed micelles may limit the rate of bioabsorption (Odeberg et al. 2003).

5. Intended Use

5.1. Method and Level of Dietary Inclusion

Cyanotech intends to market BioAstin® as an ingredient to be used in hard and soft gelatin capsules and tablets by manufacturers of human dietary supplements. Such supplements shall be formulated with an astaxanthin content of no more than 4 mg per capsule or tablet. This astaxanthin inclusion rate is the same as that used in AstaCarotene's existing Astaxin™ whole-algal product. BioAstin® was approved by the United States Food and Drug Administration as a New Dietary Ingredient and launched in 1999. BioAstin® is now marketed for use in dietary supplements in at least 20 other non-EU countries. We are aware of no reports of adverse reactions to any *H. pluvialis*-containing supplements after ten years of regular human consumption of such products at recommended astaxanthin dosages of 2-12 mg per day.

5.2. Previous Usage of Equivalent Product

Haematococcus algae have been used as a source of astaxanthin in human dietary supplements at least since 1995. In that year, the Swedish company AstaCarotene AB began marketing their Astaxin™ product in Europe. This product consisted of hard gelatin capsules containing the dried, ruptured biomass of *Haematococcus pluvialis* microalgae. AstaCarotene was later purchased by Fuji Chemical Industry Co., Ltd., of Toyama, Japan, and renamed AstaReal AB. Its Astaxin™ product, containing 4 mg astaxanthin from *Haematococcus* per capsule, is still on the market today. AstaReal also markets its bulk *Haematococcus* algae meal to supplement manufacturers under the trade name AstaCarox™. The established method and level of astaxanthin inclusion of the *Haematococcus* algae meal produced by AstaReal (AstaCarotene) are identical to those proposed for the use of BioAstin® *Haematococcus* extract in supplements.

At least two other companies have taken *Haematococcus* astaxanthin products to market as human dietary supplements: Mera Pharmaceuticals, Inc. (formerly Aquasearch, Inc.) in Kailua-Kona, Hawaii, USA, and Valensa, Inc. (formerly US Nutra LLC), in Eustis, Florida, USA. As with Cyanotech's BioAstin®, the dietary supplements produced by these companies contain a lipid extract of *Haematococcus* pluvialis algae produced using supercritical CO_2 as a solvent.

In 2004, US Nutra submitted a request to the UK Competent Authority for an opinion on the equivalence of their extracted product, Zanthin®, with AstaCarotene's whole *Haematococcus* algae meal product Astaxin™. The Competent Authority (Food Standards Agency Advisory Committee on Novel Foods and Processes) subsequently issued a ruling confirming the substantial equivalency of the extracted oleoresin with the whole algal meal.

6. Level of Undesirable Substances

6.1. Contaminants

6.1.1. Heavy Metals

Analyses of heavy metal contaminants in BioAstin® compare favorably with available data from the AstaCarox™ and Zanthin® products. All measurements are well within stated specifications and accepted safety limits.

Table 6.1.1.1. Comparative heavy metal profiles of BioAstin® Natural Astaxanthin and existing *Haematococcus* algae products

Heavy Metal Contaminant	Mean±sd ppm		
	BioAstin®*	Zanthin®†	AstaCarox™‡
arsenic	1.19±0.66	<0.5	<0.05
cadmium	<0.100	<0.5	0.01
lead	0.30±0.52	<0.5	0.03
mercury	<0.025	<0.025	<0.02

*Based on analyses of 5 lots (Covance2004d, Covance2005a, Covance2005b, Covance2002a, Covance2002b)
†Based on analysis of 1 lot (ABC Research Corporation 2003)
‡Average results (no sd given) from 3 lots (Fuji 2005)

6.1.2. Pesticides

No chemical pesticides are used at any stage of the manufacture of BioAstin®. The absence of pesticides in the product is periodically confirmed through pesticide screens carried out by Covance, Inc. (e.g., Covance2004d, Covance2002a and Covance2002b).

Negative results of pesticide screening have also been reported for the existing products Zanthin® (ABC Research Corporation 2003) and AstaCarox™ (Fuji 2005).

6.1.3. Microorganisms

Microbiological contaminants in BioAstin® are always within stated safety limits. The microbiological specifications for BioAstin are virtually identical to those for the other extracted product, Zanthin®. Higher levels of microbiological contamination are allowed by AstaCarotene (Fuji) for their whole algal product, AstaCarox™. We suspect that supplement manufacturers utilizing this product would subject it to some sort of procedure to reduce the microbial load. The supercritical CO_2 extraction process probably reduces microbial load from that of the source biomass in the BioAstin® and Zanthin® products.

Table 6.1.3.1. Comparative microbiological profiles of BioAstin® Natural Astaxanthin and existing *Haematococcus* algae products

Microbiological Contaminant	Specification cfu/g		
	BioAstin®*	Zanthin®†	AstaCarox™‡
total aerobic bacteria	$<10^3$	$<10^3$	$<10^5$
total coliforms	<10	NS§	NS
E. coli	negative	negative	$<10^2$
yeasts and molds	$<10^2$	$<10^2$	$<10^3$
Salmonella	negative	negative	negative
Staphylococcus aureus	negative	negative	$<10^2$

*Cyanotech carries out routine microbiological measurements on each lot; results are periodically validated through independent analyses by Covance, Inc. (e.g., Covance2005a and Covance2005b)
†See USNutra2004c
‡See AstaCarotene2004
§NS = not specified

6.2. Naturally Occurring Components

6.2.1. Cellular Debris

BioAstin®, being an extracted product, is substantially free of cellular debris. Although *H. pluvialis* algae meal has a history of safe consumption, the possibility exists that some rare individuals might exhibit some allergic sensitivity to cell walls and proteins. The extracted products (BioAstin®, Zanthin®) are in this regard preferable as a source of dietary astaxanthin to the existing whole algal products (Astaxin™, AstaCarox™).

6.2.2. Canthaxanthin

Haematococcus algae naturally contain small amounts of canthaxanthin. This pigment, when ingested in extremely large doses over long periods of time (e.g., as a "sunless" tanning product), may crystallize in the retina. These completely reversible retinal inclusions, which only develop at canthaxanthin doses greater than 0.2 mg/kg body weight/d, do not impair vision (Goralczyk et al. 2000). A 70 kg individual would need to consume more than 100 BioAstin® capsules per day to reach this (still safe) dosage. The canthaxanthin present in BioAstin® therefore does not represent a safety concern.

7. Other Relevant Data

7.1. Product Safety

7.1.1. History of Usage

Astaxanthin is a minor component of the human diet due to its natural presence in crustaceans and fishes, especially salmonids. A synthetic version of this pigment has been used widely in the animal feed industry as a coloring agent for the flesh of aquacultured salmon and trout, and natural sources (e.g., *Phaffia* yeast, *Haematococcus* algae) have recently been approved for this use in some countries.

The flesh of wild salmon and trout naturally contain astaxanthin in the range of 1-58 mg/kg, though for most species most of the time, the flesh astaxanthin concentration is <15 mg/kg (Torrissen et al. 1989; Turujman et al. 1997). Therefore, a daily human consumption of 300 g of wild fish flesh is equivalent to consuming no more than 17.4 mg, and more typically less than 4.5 mg, of astaxanthin. In farmed salmonids, a flesh astaxanthin level of slightly more than 4 mg/kg is usually targeted, because human visual interpretation of flesh color tends to be less sensitive to concentrations over this level compared with lower concentrations; however, in practice the farmed fish may contain up to 11.9 mg/kg of carotenoid in the flesh (Torrissen et al. 1989). Therefore, a daily human consumption of 300 g of farmed fish flesh is equivalent to consuming no more than 3.6 mg, and more typically about 1.2 mg, of astaxanthin. From these pigment levels in wild and farmed fish, we can have an expectation of safety at a daily intake of up to 17.4 mg astaxanthin, yet in practice the consumer will typically consume less than 20% of this amount.

Haematococcus algae have been used as a source of astaxanthin in human dietary supplements at least since 1995. In that year, the Swedish company AstaCarotene AB began marketing their Astaxin™ product in Europe. This product consists of hard gelatin capsules containing the dried, ruptured biomass of *Haematococcus pluvialis* microalgae. AstaCarotene was later purchased by Fuji Chemical

Industry Co., Ltd., of Toyama, Japan, and renamed AstaReal AB. Its Astaxin™ product, containing 4 mg astaxanthin from *Haematococcus* per capsule, is still on the market today. AstaReal (Fuji) also markets their dried *H. pluvialis* biomass in bulk to supplement manufacturers under the trade name AstaCarox™.

Several other companies have now taken *Haematococcus* astaxanthin products to market as human dietary supplements, including Mera Pharmaceuticals, Inc. (formerly Aquasearch, Inc.) and Cyanotech Corporation, both in Kailua-Kona, Hawaii, USA, and Valensa, Inc. (formerly US Nutra LLC), in Eustis, Florida, USA. The dietary supplements produced by these companies all contain a lipid extract of *Haematococcus* pluvialis algae produced using supercritical CO_2 as a solvent. Recommended adult dosages range from 2-12 mg of astaxanthin per day.

Human dietary supplements containing *Haematococcus* astaxanthin are marketed worldwide. For example, BioAstin®, which was approved by the United States Food and Drug Administration as a New Dietary Ingredient and launched in 1999, is now marketed for use in dietary supplements in at least 20 other non-EU countries. We are aware of no reports of adverse reactions to any *H. pluvialis*-containing supplements after ten years of regular human consumption of such products.

7.1.2. Regulatory Status

Haematococcus algae has been approved in Japan for use in both foods and animal feeds for over ten years. In August 1999, Cyanotech's *Haematococcus* algae was cleared for marketing by the US FDA as a new dietary ingredient by means of the DSHEA Act (FDA 1999). *Haematococcus*-based supplements produced by Aquasearch, US Nutra and Fuji have also been filed with FDA as new dietary ingredients. In 1999, astaxanthin from *Haematococcus* algae was approved in Canada as a feed additive pigmentation of salmonids by the Canadian Food Inspection Agency (CFIA 990535). In August 2000, astaxanthin from *Haematococcus* algae was approved by the US Food and Drug Administration as exempt from certification under 21 CFR part 73.185 (CodeFedReg2004) as a color additive in salmonid feeds.

7.1.3. Safe Handling Measures

Safety precautions for the handling of BioAstin® are the same as for ordinary vegetable oils. These precautions are primarily associated with the combustion potential of the lipid extract, and are detailed in the product Material Safety Data Sheet (Cyanotech Corporation 2003c).

7.1.4. Animal Safety Studies

7.1.4.1. F. Hoffmann-La Roche Ltd. (1987)

In this excerpt from color additive petition 7C0211, submitted to the US FDA, Hoffmann-La Roche described laboratory studies of toxicity, mutagenicity, teratology and reproductive effects associated with their synthetic astaxanthin product. Laboratory animals included rats, mice, rabbits and dogs. No adverse effects of the substance were noted, and astaxanthin was subsequently approved by FDA for use as a color additive for salmonids. Although these studies did not involve astaxanthin from *Haematococcus*, they did establish the safety of the active compound. Synthetic astaxanthin was similarly approved as a feed additive in the EU (EC no. E 161j), following the Opinion expressed by the Scientific Committee for Animal Nutrition (SCAN 1989).

7.1.4.2. Noro et al. (1988)

In this study conducted at the Animal Feeding Research Center of the Nippon Animal Feeding Corporation, Algaxan Red® *Haematococcus* algae meal was tested on mice for its acute oral toxicity. No animals died at a dosage of 18,000 mg/kg. No abnormalities in organs were noted. The study concluded that the LD_{50} for the product must be >18,000 mg/kg. [NOTE: The safety data presented here that relate to Algaxan Red® (produced by Microbio Resources, Inc.) may be considered particularly relevant to the subject of this application, BioAstin® Natural Astaxanthin. The assets of Microbio Resources, Inc., including its algal cultures and intellectual property, were acquired by Cyanotech Corporatin (MicrobioRes1996b) in 1996. Because the *Haematococcus* strain used by Microbio was the same as that now used by Cyanotech, and because of the close similarity between their respective production systems, one may consider the *Haematococcus* algae meal used for the production of BioAstin® to be equivalent to the earlier product Algaxan Red®.]

7.1.4.3. Japan Food Analysis Center (1988)

In this study conducted at the Japan Food Analysis Center, Algaxan Red® *Haematococcus* algae meal was tested for its mutagenicity, using the *Salmonella typhimurium* test. The test results were negative for mutagenicity.

7.1.4.4. Myer (1989)

In this study conducted by the International Research and Development Corporation, three lots of Algaxan Red® *Haematococcus* algae meal were tested on rats for its acute oral toxicity. All rats survived at a dosage of 5000 mg/kg, and there were no abnormalities noted in the post-mortem examinations. The study concluded that the LD_{50} for the product must be >5,000 mg/kg.

7.1.4.5. Tos et al. (1995)

In this study conducted by the Istituto di Ricerche Biomediche "Antoine Marxer" RBM S.p.A, *Haematococcus* algae meal was tested on rats for its acute oral toxicity. No animals died at a dosage of 12,000 mg/kg. No abnormalities in organs were noted. The study concluded that the LD_{50} for the product must be >12,000 mg/kg.

7.1.4.6. Yu et al. (1996)

In this study conducted by the Istituto di Ricerche Biomediche "Antoine Marxer" RBM S.p.A, *Haematococcus* algae meal was tested on rats for its repeated-dose oral toxicity over 14 days. No treatment-related deaths were noted at a daily dosage of 6,000 mg/kg. Blood chemistry and urinalysis were comparable between treatment and control groups. No compound-related changes to organs were noted during the post-mortem examination.

7.1.4.7. Edwards (1998a)

In this study conducted by Scantox DK, *Haematococcus* algae meal (produced by AstaCarotene AB) was tested for its mutagenicity, using the Ames Test with *Salmonella typhimurium* and *Eschericia coli*. The test results were negative for mutagenicity. Two independent tests using five concentrations of the test article were performed. *Haematococcus* was found to be non-mutagenic in these tests.

7.1.4.8. Edwards (1998b)

In this study conducted by Scantox DK, *Haematococcus* algae meal (produced by AstaCarotene AB) was tested for mutagenicity in a mammalian cell (mouse lymphoma) gene mutation test. Two independent tests using a range of concentrations of the test article were performed. *Haematococcus* was found to be non-mutagenic in this test.

7.1.4.9. Ono et al. (1999)

In this published study conducted at the National Institute of Health Science (Japan), an extract of *Haematococcus* algae was tested over 13 weeks for subchronic oral toxicity in rats. Three dosages were tested against a control. The highest dietary dosage was 5% *Haematococcus* extract in the feed (equivalent to 0.25% astaxanthin). No adverse effects of the test article were noted in body weight, food consumption, blood cell morphology, serum chemistry, organ weight or histopathology.

7.1.4.10 Yurkow (1999)

In this study conducted by MB Research Laboratories, *Haematococcus* algae meal (produced by Aquasearch, Inc.) was tested for 28 days for repeated oral dose toxicity in rats. A high dose group (50 mg/kg/day) and a low dose group (5 mg/kg/day) were tested against a control group (20 rats per group). The study found that the test article did not cause mortality or an increase in adverse system observations when compared to the control group. Histopathology and blood chemistry tests were in progress when the study was made public but it is unknown whether or not they were later completed.

7.1.4.11. Stewart (2001)

In this study conducted by Covance Laboratories Ltd., *Haematococcus* algae meal (produced by AstaCarotene AB) was tested on rats for oral toxicity over 13 weeks. Three dietary dosages (20%, 5% and 1%) and a control diet were administered to 20 animals per group. The study found that the *Haematococcus* algae meal was well tolerated and caused no adverse signs of toxicity. A slight increase in kidney weight was observed in animals fed the highest dosage but this was not considered to be of toxicological significance. The study concluded that an orally administered dose level of 20% represented the No Observed Adverse Effect Level (NOAEL) for the *Haematococcus* algae meal.

7.1.5. Human Safety Studies

7.1.5.1. Kuge and Silver (1999)

In this study conducted by Clinical Laboratories of Hawaii, *Haematococcus* algae meal (produced by Aquasearch, Inc.) was tested on 33 healthy adult volunteers for four weeks. One of two dosages of *Haematococcus* algae meal (3.85 or 19.25 mg astaxanthin/day) or a placebo were randomly assigned to the volunteers. The *Haematococcus* was administered in tablet form, preferably with an evening meal. Medical examination, blood chemistry analyses and urinalysis were conducted four times through the trial. The final results were reviewed by the laboratory pathologists and by the two physicians administering the examinations. No safety concerns were raised at either dosage level.

7.1.5.2. Spiller and Dewell (2003)

In this published, double-blind, placebo-controlled clinical trial, gelcaps containing an extract of *Haematococcus* algae in oil (BioAstin®, produced by Cyanotech Corporation, 2 mg astaxanthin per gelcap) were consumed by 35 healthy adult volunteers for eight weeks. The subjects consumed 3 gelcaps (either treatment or placebo) daily. Blood pressure and blood chemistry analyses were conducted three times throughout the trial. No results of any clinical significance

were observed. The study concluded that 6 mg of astaxanthin per day from *Haematococcus* algae can be safely consumed by healthy adults.

7.1.5.3. Odeberg et al. (2003)

In this published, open parallel study, the pharmacokinetics of a single large orally administered astaxanthin dose were studied in 32 healthy adult male volunteers. *Haematococcus* algae meal (produced by AstaCarotene AB) was used as a source of astaxanthin, and was administered within three different lipid compositions and in one treatment with no added lipids. All treatments contained 40 mg astaxanthin from the algae meal, and were administered in a single dose. Blood samples for determining the fate of the ingested astaxanthin were obtained initially and at ten time points out to 28 days. The study found that incorporation into lipid formulations enhanced the bioavailability of *Haematococcus* astaxanthin in humans. Astaxanthin elimination was linear, with a half-life of 15.9±5.3 hours. No safety concerns were raised by the study beyond occasional mild adverse events, which were deemed unlikely to be caused by the treatment.

7.1.5.4. Shimada et al. (2004)

In this double-blind trial, 23 healthy adult volunteers were given capsules containing 0, 2, 4 or 12 mg of astaxanthin daily for four weeks. The astaxanthin source was a *Haematococcus* extract produced by Fuji Chemical Industry Co., Ltd. Blood samples were drawn prior to supplementation and at the end of the study period. An array of clinical tests were performed on the serum samples. No dose-related changes occurred in any parameter and all values at the end of the study were within normal ranges. The study concluded that the extract of *Haematococcus* can be safely consumed by humans at astaxanthin dosages of 2-12 mg per day.

7.2. Proposal for Labeling

Labeling will adhere to applicable EU regulations for food supplements. Example labels for BioAstin® SCE10 and SCE5 may be found in Cyanotech Corporation 20006d.

7.3. Product Stability

BioAstin® displays little loss of astaxanthin during its recommended one year shelf life if maintained in the dark at <8°C. HPLC measurements of BioAstin® SCE10 have revealed losses of 5% or less during the first six months of storage.

Typically, a supplement manufacturer would incorporate this extract into gelatin capsules or beadlets soon after receiving shipment. Cyanotech has conducted stability studies on both soft gelatin capsules (Cyanotech2003d) and microencapsulated gelatin tablet-grade beadlets (Cyanotech2004c) containing

BioAstin®. Both products exhibited excellent astaxanthin stability at room temperature.

7.4. Quality Control

Samples of *Haematococcus* algae meal and BioAstin® extract are collected in sterilized bags or bottles, labeled, and transferred to the Cyanotech Quality Control Laboratory for microbiological assays, astaxanthin titer assays by HPLC and other quality control assessments. The results of all analyses are logged into worksheets and the company's Navision database. Lots failing to pass quality criteria are blocked from shipment through the Navision system and are physically quarantined. Nutritional and compositional analyses of *Haematococcus* algae meal are performed periodically on a contractual basis by Covance Laboratories Inc., Wisconsin, USA. Covance also is periodically contracted to perform tests for heavy metal, pesticide and/or microbiological contamination in the finished product.

All reasonable precautions are taken to assure that production procedures do not contribute contamination such as filth, harmful chemicals, undesirable microorganisms, or any other objectionable material to the processed product. No chemical pesticides are used in the cultivation of any of Cyanotech's microalgal products. Raw materials and ingredients are inspected and segregated as necessary to assure that they are clean, wholesome, and fit for processing and are stored under conditions that will protect against contamination and minimize deterioration. Packaging materials do not transmit contaminants or objectionable substances to the product, and provide adequate protection from contamination. All operations in receiving, inspecting, transporting, packaging, segregating, preparing, processing, and storing of the product are conducted in accord with adequate sanitation principles. Cyanotech Corporation holds a current Food Establishment Permit (DeptHealth2003) issued by the State of Hawaii for processing and packaging of food-grade algae products. Cyanotech believes that its production operations follow Current Good Manufacturing Practices as promulgated under the United States Federal Food, Drug and Cosmetic Act. The company also operates under an ISO 9001:200 (OrionReg2003) quality management system, certified annually by Orion Registrar, Inc.

Cyanotech Corporation has over 20 years of manufacturing experience with microalgal products sold into the health food, dietary supplement, medical diagnostics and animal feed industries. The company is subjected to periodic unannounced inspections from State and local representatives, and has never been cited for a major violation or issued a Form 483 violation of Good Manufacturing Practices.

7.5. Prior Opinion of the ACNFP on Extracted Oleoresin

In 2004, US Nutra LLC (now Valensa, Inc.) submitted a request to the UK Competent Authority for an opinion on the equivalence (Wilkinson2004) of their extracted product, Zanthin®, with AstaCarotene's whole *Haematococcus* algae meal supplement product Astaxin™ (in bulk known as AstaCarox™). The Competent Authority (Food Standards Agency Advisory Committee on Novel Foods and Processes or ACNFP) subsequently issued a ruling confirming the substantial equivalency (AdvCommNovFood2004) of the extracted oleoresin with the whole algal meal.

An important conclusion of the ACNFP's Opinion was that "the use of extracts from *H. pluvialis* algal meal produced by other manufacturers would be acceptable, provided that the production methods and the composition of the meal and the resulting extract were similar to those described in the [US Nutra] dossier." Cyanotech's BioAstin® and its source algae have similar compositions to US Nutra's Zanthin® and its source algae, as demonstrated through a variety of physical and chemical comparisons in the sections above. Both company's source algae are similar to that used in AstaCarotene's existing whole algal product. The extraction processes for BioAstin® and Zanthin® are nearly identical. The cultivation and initial processing steps are also similar, with the exception of a brief outdoor ripening step used by Cyanotech that is not employed by US Nutra's algal biomass supplier, Algatechnologies, Ltd. Cyanotech uses this step to achieve an economy of scale that can only be realized with large systems, which are difficult to completely enclose. At least one other *Haematococcus* astaxanthin manufacturer, Mera Pharmaceuticals, Inc., uses outdoor open systems for the brief ripening step. Moreover, it is clear that this step does not lead to adulteration or significant contamination of the product, as evidenced by the compositional, heavy metal, pesticide and microbiological contaminant assay reports described above.

We submit, therefore, that BioAstin® is essentially identical to Zanthin®, and therefore like Zanthin® it should be considered substantially equivalent to the existing AstaCarotene (AstaReal/Fuji) product Astaxin™ (AstaCarox™) in the context of Article 5 of Regulation (EC) No 258/97.

8. References

ABC Research Corp. 2003. Analytical results for Zanthin® *Haematococcus* algae extract lot no. 021003. Excerpted from: Premarket notification for a new dietary ingredient: Zanthin® Extract Astaxanthin Complex, an extract of the algae, *Haematococcus pluvialis*. United States Food and Drug Administration, Docket 95S-0316, RPT237. 4 pp.

Advisory Committee on Novel Foods and Processes. 2004. Request for an Article 5 opinion on the substantial equivalence of astaxanthin-rich carotenoid oleoresin extracted from *Haematococcus pluvialis*. UK Food Standards Agency. 4 pp.

Aoi, W., Y. Naito, K. Sakuma, M. Kuchide, H. Tokuda, T. Maoka, S. Toyokuni, S. Oka, M. Yasuhara, and T. Yoshikawa. 2003. Astaxanthin limits exercise-induced skeletal and cardiac muscle damage in mice. Antioxidants & Redox Signaling 5: 139-144.

AstaCarotene AB. 2004. Specification sheet for AstaCarox™ *Haematococcus* algae meal. Excerpted from: Premarket notification for a new dietary ingredient: Zanthin® Extract Astaxanthin Complex. United States Food and Drug Administration, Docket 95S-0316, RPT278. 1 p.

Baker, R., and C. Günther. 2004. The role of carotenoids in consumer choice and the likely benefits from their inclusion into products for human consumption. Trends in Food Science & Technology 15: 484-488.

Bjerkeng, B. 1997. Chromatographic analysis of synthesized astaxanthin – a handy tool for the ecologist and the forensic chemist? The Progressive Fish-Culturist 59: 129-140.

Bjerkeng, B. 2000. Carotenoid pigmentation of salmonid fishes – recent progress. In: Cruz -Suárez, L.E., Ricque-Marie, D., Tapia-Salazar, M., Olvera-Novoa, M.A. y Civera-Cerecedo, R., (Eds.). Avances en Nutrición Acuícola V. Memorias del V Simposium Internacional de Nutrición Acuícola. 19-22 Noviembre, 2000. Mérida, Yucatán, pp. 71-89.

Boussiba, S., L. Fan, and A. Vonshak. 1992. Enhancement and determination of astaxanthin accumulation in green alga *Haematococcus pluvialis*. Methods in Enzymology 213: 386-391.

Buchwald, M., and W.P. Jencks. 1968. Optical properties of astaxanthin solutions and aggregates. Biochemistry 7: 834-843.

Canadian Food Inspection Agency. 2005. *Haematococcus* algae meal, comminuted, dehydrated (or Dried *Haematococcus* algae meal). CFIA Feed Section Schedule IV, Part II, No. 8.63. 12 pp.

Chew, B.P., M.G. Hayek, and J.S. Park. 2004a. Methods and compositions utilizing astaxanthin. United States Patent Application Publication No. US 2004/0151761 A1. 25 pp.

Chew, B.P., J.S. Park, M.G. Hayek, S.P. Massimino, and G.A. Reinhart. 2004b. Astaxanthin is taken up by blood lipoproteins and leukocytes in dogs and cats. 2004 Experimental Biology meeting abstracts [accessed at http://select.biosis.org/faseb]. FASEB Journal 18: A158, Abstract #131.13.

Chew, B.P., J.S. Park, M.G. Hayek, S.P. Massimino, and G.A. Reinhart. 2004c. Cell-mediated and humoral immune response in dogs fed astaxanthin. 2004 Experimental Biology meeting abstracts [accessed at http://select.biosis.org/faseb]. FASEB Journal 18: A533, Abstract #367.5.

Chew, B.P., J.S. Park, M.G. Hayek, S.P. Massimino, and G.A. Reinhart. 2004d. Dietary astaxanthin stimulates cell-mediated and humoral immune response in cats. 2004 Experimental Biology meeting abstracts [accessed at http://select.biosis.org/faseb]. FASEB Journal 18: A533, Abstract #367.6.

Cifuentes, A.S., M.A. Gonzáles, S. Vargas, M. Hoeneisen, and N. González. 2003. Optimization of biomass, total carotenoids and astaxanthin production in *Haematococcus pluvialis* Flotow strain Steptoe (Nevada, USA) under laboratory conditions. Biological Research 36: 343-357.

Code of Federal Regulations. 2004. *Haematococcus* algae meal. 21 CFR 73.185. United States Government Printing Office. 2 pp.

CONFIDENTIAL. 2006. Supercritical CO_2 extraction process for BioAstin Natural Astaxanthin. 2 pp.

Coral-Hinostroza, G.N., T. Ytrestøyl, B. Ruyter, and B. Bjerkeng. 2004. Plasma appearance of unesterified astaxanthin geometrical *E/Z* and optical *R/S* isomers in men given single doses of a mixture of optical 3 and 3'*R/S* isomers of astaxanthin fatty acyl diesters. Comparative Biochemistry and Physiology Part C 139: 99-110.

Covance Laboratories Inc. 2002a. Report of analysis: BioAstin® lot no. 1005000023. Madison, Wisconsin, USA. 6 pp.

Covance Laboratories Inc. 2002b. Report of analysis: BioAstin® lot no. 1005020008. Madison, Wisconsin, USA. 6 pp.

Covance Laboratories Inc. 2004a. Report of analysis: NatuRose® lot no. 1004000726. Madison, Wisconsin, USA. 10 pp.

Covance Laboratories Inc. 2004b. Report of analysis: NatuRose® lot no. 1004000731. Madison, Wisconsin, USA. 7 pp.

Covance Laboratories Inc. 2004c. Report of analysis: NatuRose® lot no. 1004000732. Madison, Wisconsin, USA. 7 pp.

Covance Laboratories Inc. 2004d. Report of analysis: BioAstin® lot no. 1005010384. Madison, Wisconsin, USA. 6 pp.

Covance Laboratories Inc. 2005a. Report of analysis: BioAstin® lot no. 1005010576. Madison, Wisconsin, USA. 5 pp.

Covance Laboratories Inc. 2005b. Report of analysis: BioAstin® lot no. 1005010674. Madison, Wisconsin, USA. 7 pp.

Cronin, J.R. 2002. The potential of astaxanthin: A new multipurpose, high-power antioxidant. Alternative & Complementary Therapies 8: 44-49.

Cyanotech Corporation. 2003a. HPLC and spectrophotometric analysis of carotenoids from *Haematococcus* algae powder (NatuRose®). Cyanotech Corporation Technical Bulletin Ax-015. 11 pp.

Cyanotech Corporation. 2003b. HPLC and spectrophotometric analysis of carotenoids from *Haematococcus* algae oleoresin (BioAstin® SCE) with supplemental instructions included for gelcaps. Cyanotech Corporation Technical Bulletin Ax-020. 11 pp.

Cyanotech Corporation. 2003c. Material Safety Data Sheet: BioAstin® SCE10 Natural Astaxanthin. Cyanotech Corporation Technical Bulletin Ax-079. 3 pp.

Cyanotech Corporation. 2003d. Stability trial of gelcaps containing BioAstin® Natural Astaxanthin. 1 p.

Cyanotech Corporation. 2004b. Thin-layer chromatography (TLC) system for NatuRose® carotenoids. Cyanotech Corporation Technical Bulletin Ax-003. 4 pp.

Cyanotech Corporation. 2004c. Stability trial of tablet-grade beadlets containing BioAstin® Natural Astaxanthin. 1 p.

Cyanotech Corporation. 2004d. Chromatograms from HPLC analyses of three lots of *Haematococcus* algae meal. 3 pp.

Cyanotech Corporation. 2006a. HPLC analyses of four lots of BioAstin® SCE10 Natural Astaxanthin. 7 pp.

Cyanotech Corporation. 2006b. HPLC analyses of three lots of BioAstin® SCE5 Natural Astaxanthin. 6 pp.

Cyanotech Corporation. 2006c. Schematic flowchart of production process for BioAstin® Natural Astaxanthin. 1 p.

Cyanotech Corporation. 2006d. Example labels for BioAstin® SCE10 and SCE5 Natural Astaxanthin products. 2 p.

Department of Health. 2003. Food Establishment Permit for Cyanotech Corporation. State of Hawaii, USA. 1p.

Dodd, J.C. 1986. Elements of pond design and construction. In: Richmond, A. [ed], CRC Handbook of Microalgal Mass Culture, CRC Press, Boca Raton, Florida, USA. pp. 265-283.

Dore, J.E. 2005. Astaxanthin and cancer chemoprevention. In: Bagchi, D., and H.G. Preuss [eds], Phytopharmaceuticals in Cancer Chemoprevention. CRC Press, Boca Raton, Florida, USA. pp. 555-573.

Edwards, C.N. 1998a. *Haematococcus pluvialis*: Ames test "treat and plate test". Scantox, Report No. 28708. Lille Skensved, Denmark. Excerpted from: Premarket notification for a new dietary ingredient: Astaxanthin, extracted from the *Haematococcus pluvialis* algae. United States Food and Drug Administration, Docket 95S-0316, RPT236. 19 pp.

Edwards, C.N. 1998b. *Haematococcus pluvialis*: *In vitro* mammalian cell gene mutation test performed with mouse lymphoma cells (L5178Y). Scantox, Report No. 28709. Lille Skensved, Denmark. Excerpted from: Premarket notification for a new dietary ingredient: Astaxanthin, extracted from the *Haematococcus pluvialis* algae. United States Food and Drug Administration, Docket 95S-0316, RPT236. 15 pp.

F. Hoffman-La Roche Ltd. 1987. Astaxanthin: human food safety summary. Excerpted from: Astaxanthin as a pigmenter in salmon feed, Color Additive Petition 7C0211, United States Food and Drug Administration. 9 pp.

FEEDAP. 2005. Opinion of the Scientific Panel on Additives and Products or Substances used in Animal Feed on the request from the European Commission on the safety of use of colouring agents in animal nutrition. Part I. General principles and astaxanthin. EFSA Journal 291: 1-40.

Food and Drug Administration. 1999. Acknowledgement of premarket notification by Cyanotech Corporation for *Haematococcus* pluvialis algae as a new dietary ingredient. Excerpted from: Premarket notification for a new dietary ingredient: *Haematococcus pluvialis* algae. United States Food and Drug Administration, Docket 95S-0316, RPT50. 2 pp.

Fuji Chemical Industry Co., Ltd. 2005. Typical analysis results for AstaCarox™ V1010 *Haematococcus* algae meal. Excerpted from: Premarket notification for a new dietary ingredient: Astaxanthin present in *Haematococcus pluvialis* algal biomass. United States Food and Drug Administration, Docket 95S-0316, RPT274. 1 p.

Grung, M., F.M.L. D'Souza, M. Borowitzka, and S. Liaaen-Jensen. 1992. Algal carotenoids 51. Secondary carotenoids 2. *Haematococcus pluvialis* aplanospores as a source of (3S, 3'S)-astaxanthin esters. Journal of Applied Phycology 4: 165-171.

Goralczyk, R., F.M. Barker, S. Buser, H. Liechti, and J. Bausch. 2000. Dose dependency of canthaxanthin crystals in monkey retina and spatial distribution of its metabolites. Investigative Ophthalmology & Visual Science 41: 1513-1522.

Guerin, M., M.E. Huntley, and M. Olaizola. 2003. *Haematococcus* astaxanthin: applications for human health and nutrition. Trends in Biotechnology 21: 210-216.

Hazen, T.E. 1899. The life history of *Sphaerella lacustris* (*Haematococcus pluvialis*). Memoirs of the Torrey Botanical Club 6: 211-247.

Jacobs, P.B., R.D. LeBoeuf, S.A. McCommas, and J.D. Tauber. 1982. The cleavage of carotenoid esters by cholesterol esterase. Comparative Biochemistry and Physiology 72B: 157-160.

Japan Food Analysis Center. 1988. Mutagenicity test of alga meal. JFAC Report No. 41020585. Japan. Excerpted from: *Haematococcus* algae meal as a pigmenter for aquaculture feeds, Color Additive Petition 1C0237, United States Food and Drug Administration. 8 pp.

Kistler, A., H. Liechti, L. Pichard, E. Wolz, G. Oesterhelt, A. Hayes, and P. Maurel. 2002. Metabolism and CYP-inducer properties of astaxanthin in man and primary human hepatocytes. Archives of Toxicology 75: 665-675.

Kuge, M., and A. Silver. 1999. A human safety trial of natural astaxanthin from *Haematococcus pluvialis* algae. Clinical Laboratories of Hawaii, Honolulu and Kailua-Kona, Hawaii, USA. Excerpted from: Premarket notification for a new dietary ingredient: *Haematococcus pluvialis* algae. United States Food and Drug Administration, Docket 95S-0316, RPT65. 29 pp.

Kurihara, H., H. Koda, S. Asami, Y. Kiso, and T. Tanaka. 2002. Contribution of the antioxidative property of astaxanthin to its protective effect on the promotion of cancer metastasis in mice treated with restraint stress. Life Sciences 70: 2509-2520.

Lorenz, R.T., and G.R. Cysewski. 2000. Commercial potential for *Haematococcus* microalgae as a natural source of astaxanthin. Trends in Biotechnology 18: 160-167.

Maher, T.J. 2000. Astaxanthin continuing education module. New Hope Institute of Retailing, Boulder, Colorado, USA. 8 pp.

Melkonian, M. 1989. Phylum Chlorophyta, class Chlorophyceae. In: Margulis, L., J.O. Corliss, M. Melkonian, D.J. Chapman, and H.I. McKhann [eds], Handbook of Protoctista, Jones and Bartlett Publishers Inc., Boston, Massachusetts, USA. pp. 608-616.

Microbio Resources Inc. 1996b. Correspondence with Cyanotech Corporation documenting the sale of Microbio's assets to Cyanotech. 2 pp.

Myer, J.R. 1989. Algae meal: acute oral toxicity study in rats. International Research and Development Corporation, Report No. 538-002. Mattawan, Michigan, USA. Excerpted from: *Haematococcus* algae meal as a pigmenter for aquaculture feeds, Color Additive Petition 1C0237, United States Food and Drug Administration. 17 pp.

Naguib, Y. 2001. Pioneering astaxanthin. Nutrition Science News 6: 58-62.

Newly Weds Foods Co. 2005. Product data sheet for NatureGuard™ Rosemary Extract O. 2 pp.

Noro, H., T. Shimizu, S. Yonemochi, Y. Kazahaya, and H. Yamazaki. 1988. Acute toxicity test of alga meal. Animal Feeding Research Center, Nippon Animal Feeding Corporation. Japan. Excerpted from: *Haematococcus* algae meal as a pigmenter for aquaculture feeds, Color Additive Petition 1C0237, United States Food and Drug Administration. 4 pp.

Odeberg, J.M., Å. Lignell, A. Pettersson, and P. Höglund. 2003. Oral bioavailability of the antioxidant astaxanthin in humans is enhanced by incorporation of lipid based formulations. European Journal of Pharmaceutical Sciences 19: 299-304.

Oilseeds International, Ltd. 2004. Product data sheet for California High Oleic Safflower Oil. 2 pp.

Olaizola, M., and M.E. Huntley. 2003. Recent advances in commercial production of astaxanthin from microalgae. In: Fingerman, M., and R. Nagabhushanam [eds], Recent Advances in Marine Biotechnology, Vol. 9: Biomaterials and Bioprocessing, Science Publishers Inc., Enfield, New Hampshire, USA. pp. 143-164.

Ono, A., K. Sekita, M. Saitoh, T. Umemura, Y. Ogawa, T. Furuya, T. Kaneko, and T. Inoue. 1999. A 13-week subchronic oral toxicity study of *Haematococcus* color in F334 rats. Bulletin National Health Science 117: 91-98.

Orion Registrar Inc. 2003. Certificate of Registration of ISO 9001:2000 Quality Management System for Cyanotech Corporation. Arvada, Colorado, USA. 1p.

Østerlie, M., B. Bjerkeng, and S. Liaaen-Jensen. 1999. Accumulation of astaxanthin all-E, 9Z and 13Z geometrical isomers and 3 and 3' RS optical isomers in rainbow trout (*Oncorhynchus mykiss*) is selective. Journal of Nutrition 129: 391-398.

Østerlie, M., B. Bjerkeng, and S. Liaaen-Jensen. 2000. Plasma appearance and distribution of astaxanthin *E/Z* and *R/S* isomers in plasma lipoproteins of men after single dose administration of astaxanthin. Journal of Nutritional Biochemistry 11: 482-490.

Rao, A.V., and S. Agarwal. 2000. Role of antioxidant lycopene in cancer and heart disease. Journal of the American College of Nutrition 19: 563-569.

Renstrøm, B., G. Borch, O.M Skulberg, and S. Liaaen-Jensen. 1981. Optical purity of (3S,3'S)-astaxanthin from *Haematococcus pluvialis*. Phytochemistry 20: 2561-2564.

Schüep, W., and J. Schierle. 1995. Example 9: Astaxanthin. Determination of stabilized, added astaxanthin in fish feeds and pre-mixes. In: Britton, G., S. Liaaen-Jensen, and H. Pfander [eds], Carotenoids, Vol. 1A: Isolation and Analysis. Birkhäuser Verlag, Basel, Switzerland. pp. 273-276.

Scientific Committee for Animal Nutrition. 1989. Report of the Scientific Committee for Animal Nutrition on the use of astaxanthin in feedingstuffs for salmon and trout. European Union. 4 pp.

Shao, A. 2001. The role of lutein in human health. Journal of the American Nutraceutical Association 4: 8-19.

Shimada, et al. 2004. Safety study of astaxanthin consumption in humans. Fujita Health University, Toyoake, Japan. Excerpted from: Premarket notification for a new dietary ingredient: Astaxanthin, extracted from the *Haematococcus pluvialis* algae. United States Food and Drug Administration, Docket 95S-0316, RPT236. 6 pp.

Showalter, L.A., S. A. Weinman, M. Østerlie, and S.F. Lockwood. 2004. Plasma appearance and tissue accumulation of non-esterified, free astaxanthin in C57BL/6 mice after oral dosing of a disodium disuccinate diester of astaxanthin (*Heptax*™). Comparative Biochemistry and Physiology Part C 137: 227-236.

Spencer, K.G. 1989. Pigmentation supplements for animal feed compositions. United States Patent No. 4,871,551. 7 pp.

Spiller, G.A., and A. Dewell. 2003. Safety of an astaxanthin-rich *Haematococcus pluvialis* algal extract: a randomized clinical trial. Journal of Medicinal Food 6: 51-56.

Stewart, J.S. 2001. *Haematococcus pluvialis* powder: 13 week oral (dietary administration) toxicity study in the rat. Covance Laboratories Ltd., Report No. 1840/002. North Yorkshire, United Kingdom. Excerpted from: Premarket notification for a new dietary ingredient: Astaxanthin, extracted from the *Haematococcus pluvialis* algae. United States Food and Drug Administration, Docket 95S-0316, RPT236. 41 pp.

Torrissen, O.J., R.W. Hardy, and K.D. Shearer. 1989. Pigmentation of salmonids – carotenoid deposition and metabolism. Reviews in Aquatic Sciences 1: 209-225.

Tos, E.G., R. Maraschin, L. Orlando, P. Zaninelli, and B. Piccioli. 1995. *Haematococcus pluvialis*, unicellular green algae: Acute toxicity study in rats treated by oral route. Istituto di Ricerche Biomediche "Antoine Marxer" RBM S.p.A., Report No. 950053. Torino, Italy. Excerpted from: *Haematococcus* algae astaxanthin, Food Additive Petition 2A4732, United States Food and Drug Administration. 19 pp.

Turujman, S.A., W.G. Wamer, R.R. Wei, and R.H. Albert. 1997. Rapid liquid chromatographic method to distinguish wild salmon from aquacultured salmon fed synthetic astaxanthin. Journal of AOAC International 80: 622-632.

US Nutra LLC. 2004a. Fatty acid and carotenoid data for *Haematococcus* algae meal produced by Algatechnologies and Mera Pharmaceuticals. Excerpted from: Premarket notification for a new dietary ingredient: Zanthin® Extract Astaxanthin Complex, an extract of the algae, *Haematococcus pluvialis*. United States Food and Drug Administration, Docket 95S-0316, RPT237. 1 p.

US Nutra LLC. 2004b. Fatty acid and carotenoid data for Zanthin® *Haematococcus* algae extract compared with *Haematococcus* algae meal. Excerpted from: Premarket notification for a new dietary ingredient: Zanthin® Extract Astaxanthin Complex, an extract of the algae, *Haematococcus pluvialis*. United States Food and Drug Administration, Docket 95S-0316, RPT237. 1 p.

US Nutra LLC. 2004c. Product specification sheet for Zanthin® *Haematococcus* algae extract. Excerpted from: Premarket notification for a new dietary ingredient: Zanthin® Extract Astaxanthin Complex, an extract of the algae, *Haematococcus pluvialis*. United States Food and Drug Administration, Docket 95S-0316, RPT237. 1p.

US Nutra LLC. 2004d. Production diagram and extraction procedure for Zanthin® Astaxanthin-Complex Carotenoid Oleoresin. Excerpted from: Premarket notification for a new dietary ingredient: Zanthin® Extract Astaxanthin Complex, an extract of the algae, *Haematococcus pluvialis*. United States Food and Drug Administration, Docket 95S-0316, RPT237. 2p.

Vecchi, M., E. Glinz, V. Meduna, and K. Schiedt. 1987. HPLC separation and determination of astacene, semiastacene, astaxanthin, and other keto-carotenoids. Journal of High-Resolution Chromatography and Chromatography Communications 10: 348-351.

Wiener, H., A. Drory, and L. Line. 2003. Astaxanthin from the microalga *Haematococcus* – a superb natural antioxidant for human health. Innovations in Food Technology (November 2003): 28-32

Wilkinson, J. 2004. Substantial equivalence application for the approval of an astaxanthin-rich carotenoid oleoresin, Zanthin® derived from *Haematococcus pluvialis* for use in dietary supplement tablets and capsules. Written and submitted to the UK Food Standards Agency on behalf of US Nutra LLC, Eustis, Florida, USA. 25 pp.

Wolz, E., H. Liechti, B. Notter, G. Oesterhelt, and A. Kistler. 1999. Characterization of metabolites of astaxanthin in primary cultures of rat hepatocytes. Drug Metabolism and Disposition 27: 456-462.

Yu, P., R. Maraschin, L. Orlando, G. Oberto, S. Peano, M. Carbonatto, P. Zaninelli, B. Piccioli, and G. Peretti. 1996. *Haematococcus pluvialis*, unicellular green algae:14-day oral toxicity study in rats. Istituto di Ricerche Biomediche "Antoine Marxer" RBM S.p.A., Report No. 950501. Torino, Italy. Excerpted from: *Haematococcus* algae astaxanthin, Food Additive Petition 2A4732, United States Food and Drug Administration. 31 pp.

Yurkow, E.J. 1999. *Haematococcus pluvialis*: 28-day repeated dose oral toxicity study in rats. MB Research Laboratories, Report No. MB 98-7853.01. Spinnerstown, Pennsylvania, USA. Excerpted from: Premarket notification for a new dietary ingredient: *Haematococcus pluvialis* algae. United States Food and Drug Administration, Docket 95S-0316, RPT65. 35 pp.

Zhekisheva, M., S. Boussiba, I. Khozin-Goldberg, A. Zarka, and Z. Cohen. 2002. Accumulation of oleic acid in *Haematococcus pluvialis* (Chlorophyceae) under nitrogen starvation or high light is correlated with that of astaxanthin esters. Journal of Phycology 38: 325-331.