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VINCENT A. KLEINFELD
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ALAN H. KAPLAN
1930-2001

March 25, 2004

**CONFIRMATION
COPY**

Victoria L. Lutwak
Office of Nutritional Products Labeling and Dietary Supplements
Center for Food Safety and Applied Nutrition
Food and Drug Administration (HFS-822)
5100 Paint Branch Parkway
College Park, MD 20740

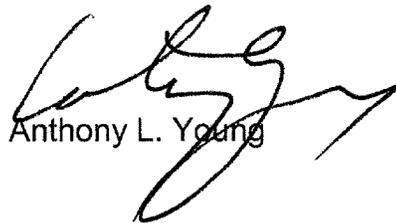
Re: New Dietary Ingredient Notification for U.S. Nutraceuticals

Dear Ms. Lutwak;

Following up on your telephone call yesterday afternoon, the name of the new dietary ingredient that is the subject of the U.S. Nutraceuticals premarket notification is ZANTHINE Extract Astaxanthin Complex - 10% Standardized. This is an extract of *Haematococcus pluvialis* Flotow emend. Wille

Thank you again for your call and please contact us if you have any further questions.

Sincerely yours,


Anthony L. Young

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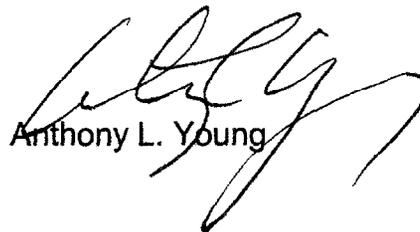
Office of Nutritional Products,
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Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740

MAR 24 2004
A.B./FDA

Dear Sir/Madam

Pursuant to Section 413(a) of the Federal Food, Drug, and Cosmetic Act and 21 C.F.R. Section 190.6, please accept for filing the enclosed original and two copies of a New Dietary Ingredient Notification for U.S. Nutraceuticals, LLC, Eustis, Florida. Please call, write or e-mail the undersigned (ayoung@kkblaw.com), who is designated by U.S. Nutraceuticals, should you have any questions regarding this submission.

Sincerely yours,


Anthony L. Young

Enclosures

Lutwak, Vickey

From: Anthony L. Young [ayoung@kkblaw.com]
Sent: Thursday, March 25, 2004 11:05 AM
To: Lutwak, Vickey; Lutwak, Vickey
Subject: U.S. Nutraceuticals

I just pdf'd the letter to you responding to your questions. A hard copy will be sent regular mail.

Tony Young

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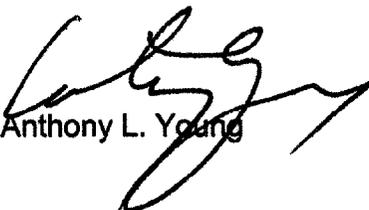
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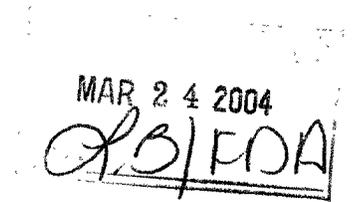
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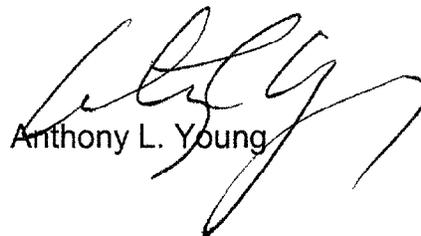
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Dear Sir/Madam

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Sincerely yours,


Anthony L. Young

Enclosures

**New Dietary Ingredient Notification
For Astaxanthin-Rich Carotenoid Oleoresin
Extracted from *Haematococcus pluvialis***

I. Introduction

U.S. Nutraceuticals hereby submits this New Dietary Ingredient Notification, for the use in dietary supplements of astaxanthin-rich carotenoid oleoresin (extracted from the freshwater algae, *Haematococcus pluvialis*), to the U.S. Food and Drug Administration (FDA) pursuant to section 413(a) of the Federal Food, Drug, and Cosmetic Act and 21 CFR § 190.6. U.S. Nutraceuticals intends to produce and market this astaxanthin product as ZANTHIN® Extract Astaxanthin Complex – 10% Standardized.

U.S. Nutraceuticals' address is 2751 Nutra Lane, Eustis, Florida 32726.

It is the position of U.S. Nutraceuticals that *Haematococcus pluvialis* is a well documented, safe source of astaxanthin-rich carotenoid oleoresin and that the extract is reasonably expected to be safe for use in dietary supplements for levels of intake of up to five milligrams per day (5 mg/day) of astaxanthin.

Safety-related data for astaxanthin were reviewed by FDA in connection with the approval of astaxanthin as a feed additive for pigmentation in salmonid fish, which was submitted on August 11, 1987 by Roche Vitamins and Fine Chemicals, a division of Hoffman-La Roche, Inc., and is promulgated under 21 C.F.R. § 73.35. Also, U.S. Nutraceuticals notes that FDA, in connection with the submission of New Dietary Ingredient Notifications for astaxanthin-related products, filed in Docket No. 95S-0316 by Neptune Technologies & Bioresources (RPTs 131, 132, 133, and 145), Micro Gaia, Inc. (RPT 119), Igene Biotechnology, Inc. (RPT 74), Aquasearch, Inc. (RPT 65), and Cyanotech Corp. (RPTs 45 and 50), has ultimately filed these notifications without adverse comments.

U.S. Nutraceuticals will begin marketing its ZANTHIN® Extract Astaxanthin Complex – 10% Standardized product 75 days after acknowledgement of FDA's receipt of this notification unless otherwise instructed by the agency.

II. Product Description

A. General Background – *Haematococcus pluvialis* Algae

U.S. Nutraceuticals intends to produce ZANTHIN® Extract Astaxanthin Complex – 10% Standardized, an astaxanthin-rich carotenoid oleoresin, from *Haematococcus pluvialis* algae, which is a well-documented, safe source of astaxanthin. *Haematococcus pluvialis*, also referred to as *Haematococcus lacustris* or *Sphaerella lacustris*, is a ubiquitous green alga, which is classified as follows:

Classification

Phylum:	Chlorohyta
Class:	Chlorophyceae
Order:	Volvocales
Family:	Haematococcaceae
Genus:	Haematococcus
Species:	pluvialis

The alga occurs in nature throughout the world where environmental conditions for its growth are favorable. *Haematococcus* algae has a number of different forms during its life cycle and is normally found in ephemeral pools of relatively cool fresh water. Under these conditions, *Haematococcus* is a green motile alga about 20-30 µm in size that utilizes the available nitrate, phosphate, and other nutrients to grow and reproduce (i.e., “Green Stage”). When nutrients or water become limiting, the alga forms a protective cell wall and encyst and enters a dormant phase. During this dormant phase, large amounts of astaxanthin are produced, turning the cells red (i.e., “Red Stage”). Alga cells can remain viable in the encysted stage for decades but return to the motile phase upon the reintroduction of adequate nutrients and water.

In the commercial production process, the Green Stage starts indoors with a single-cell colony of *Haematococcus* algae and continues outdoors in solar-powered photobioreactors. The purpose of this stage is to produce large numbers of viable, unstressed algal cells via the normal cell division process. The Red Stage is initiated by subjecting the cells to severe stress conditions through high radiation intensity and changes in growth media. As a result, cells with high astaxanthin levels (up to 4% of the cell’s dry weight) are produced in a closed, environmentally controlled process that limits biological and chemical contamination.¹

B. General Background – Astaxanthin

Astaxanthin, known chemically as 3,3'-dihydroxy- β,β -carotene-4,4'-dione, is a member of the carotenoid family, which consists of over 700 natural, lipid-soluble pigment primarily produced by aquatic animals and unicellular organisms such as yeast and algae. Most notably, in yeast and algae, carotenoids serve as secondary light-harvesting pigment in the photosynthetic process. Carotenoids are synthesized through the isoprenoid biosynthetic pathway, which is also responsible for such diverse compounds as prostaglandins, steroids, sterols, and vitamins A, D, E and K. The pathway begins with acetyl-Co-A and proceeds through phytoene, lycopene, β -carotene, and canthaxanthin before the last oxidative steps result in astaxanthin (see Appendix A).

Unsubstituted carotenoids are called carotenes (i.e., β -carotene). In contrast, carotenoids with hydroxy, keto, methoxy, epoxy, or carboxyl groups are collectively known as xanthophylls. Mono- and diesters of astaxanthin derive from the esterification of fatty acids to the 3 or 3' hydroxyl groups, resulting in a molecule that is more soluble in the cellular environment and inherently more stable to oxidation.

Astaxanthin has been found to be the most widely distributed and major carotenoid found in marine fish and shellfish.² Because only plants and microalgae are able to synthesize carotenoids, animals must obtain carotenoids from their diets. Relative to other carotenoids studied, astaxanthin has been found in large amounts in the pollack, Pacific cod, Chum salmon, flounder, blue and snow crab, lobster, shrimp, and prawn. Astaxanthin is also found as a pigment throughout the animal kingdom, including in the plumage of flamingoes and cedar waxwings, in the combs of roosters, and in the retinas of shore birds.

C. Technical Description of Astaxanthin

The following are the principal chemical attributes of astaxanthin:

- Chemical name 3,3'-dihydroxy- β,β -carotene-4,4'-dione
- Primary synonyms 3',3'-dihydroxy-4,4'-diketo- β -carotene; 3,3'-dihydroxy-[all-trans-1,18-(3,7,12, 16-tetramethyl-1,3,5,7,9,11,13,15,17-octadecanonaen-1,18-diyl)-bis-(2,6,6-trimethyl-cyclohexen)]-4,4' dione

U.S. Nutraceuticals, LLC

- Molecular formula $C_{40}H_{53}O_4$
- Molecular weight 596.86
- Molecular structure See Appendix B
- CAS number 7542-45-2
- EINECS number 207-451-4

The astaxanthin molecule has two asymmetric carbons located at the 3 and 3' positions of the benzenoid rings on both ends of the molecule (see Appendix B). The positioning of the hydroxyl groups (-OH) on these carbon atoms result in three different enantiomers of astaxanthin, which are designated R,R', S,S', and R,S'(meso). Free astaxanthin and the mono- and diesters of astaxanthin from *Haematococcus pluvialis* algae are comprised solely of the optically pure (S,S') enantiomer.

D. U.S. Nutraceuticals' Astaxanthin-Complex Carotenoid Oleoresin

U.S. Nutraceuticals's ZANTHIN® Extract Astaxanthin Complex – 10% Standardized is produced from the freshwater algae *Haematococcus pluvialis*. The feedstock algae used for extraction by U.S. Nutraceuticals is identical to those products (used in unpurified form) for which New Dietary Ingredient Notifications have been submitted to FDA by Cyanotech Corporation and Aquasearch Inc.

The oleoresin production consists of extracting the oil and oil-soluble components from the dry algae using supercritical carbon dioxide as a clean solvent (i.e., no organic solvents or any type of entrainers). This extraction technology is currently utilized to extract caffeine from coffee beans and flavors from hops and food spices. A description of the extraction process utilized by U.S. Nutraceuticals and a diagram of the extraction procedure as it applies to astaxanthin-complex carotenoid oleoresin is attached as Appendix C.

Supercritical carbon dioxide extracts oil and carotenoids in the relative proportions found in the algae and does not modify naturally occurring compounds or leave any lasting solvent in the extract or residue. Dry *Haematococcus pluvialis* algae biomass used for extraction has a typical composition of:

- Protein 17 – 25%
- Carbohydrates 30 – 40%
- Ash 2 – 4%
- Lipids 20 – 35%

U.S. Nutraceuticals, LLC

- Moisture 3 – 10%
- Carotenoids 2 – 4%

The resulting supercritical oleoresin extract contains only the original existing non-polar lipids, carotenoids, and water. Greater than 85 percent of the carotenoid complex is composed of astaxanthin in the free, mono-, and diester forms that are present in the algae, with the remainder consisting of lutein, β -carotene, and other carotenoids. Also, as in the algae, the astaxanthin is comprised entirely of optically pure 3S, 3S' isomer.

The purpose of the extraction procedure is to efficiently recover the carotenoids contained in the algae. The recovery rate of astaxanthin is in excess of 90 percent of the amount in the algae. The processing byproduct (not used by U.S. Nutraceuticals) is a defatted algae powder comprised of the protein, carbohydrates, ash, and any extraneous microbial load originally present in the dried algae raw material.

The product specifications for the product U.S. Nutraceuticals intends to market, ZANTHIN® Extract Astaxanthin Complex – 10% Standardized, are attached as Appendix D. The analytical method used to determine the composition of astaxanthin complex is attached as Appendix E. The results from a full compositional, heavy metal, and pesticide screening of a typical batch of ZANTHIN® Extract Astaxanthin Complex – 10% Standardized are attached as Appendix F.

Stability studies have been performed on ZANTHIN® Extract Astaxanthin Complex – 10% Standardized and other products in which it is used as an ingredient. As summarized in Appendix G, these studies support a shelf life for ZANTHIN® Extract Astaxanthin Complex – 10% Standardized of fourteen months from the date of manufacture when stored refrigerated at 2 – 8°C. Also, room temperature storage of the product, while not recommended, is supported at 60 days under ambient conditions. Furthermore, short-term use of higher temperatures (i.e., greater than 40°C for the production of other dietary supplements) has not been shown to cause degradation of the product. Accelerated stability studies performed on the astaxanthin complex in 2.5% compressible beadlets, 2.0% cold water dispersible beadlets, and softgels (1 mg) demonstrated a decline in astaxanthin complex concentration of less than 5% after four weeks and a maximum decline of 8.4% after six weeks in the each of the beadlet products and no decline in astaxanthin complex concentration after three months in the softgel. Real time stability studies on these products are continuing.

E. Mechanism of Action of Astaxanthin

Astaxanthin, a xanthophyll member of the carotenoid family, is fat-soluble due to its polar end groups. After ingestion, astaxanthin is absorbed by the duodenal mucosa via passive or simple diffusion and transported to the liver by the lymph system. In the liver, a lipoprotein is attached to the molecule, which aids its distribution throughout the body via blood.

In cells, the polarity of the astaxanthin molecule allows it to span the lipid bilayer and become embedded in the cell membrane, the site where attack by free radicals initially occurs. Astaxanthin traps an alkoxy radical in the central hydrophobic region of the lipid bilayer and transports the unpaired electron of the free radical along its conjugated polyene structure to the fat/water interface. At this interface, the resonance-stabilized astaxanthin radical reacts with a water-soluble scavenger, such as ascorbic acid, and renders it harmless. Only carotenoids with a 4-carbon group (i.e., astaxanthin) can facilitate this reaction, and astaxanthin can trap two radicals per molecule. Because the molecular weight of astaxanthin is under 600 Daltons (596.8 Da) and it is a lipophilic molecule, it is able to cross the blood/brain barrier, where it may serve as a potent antioxidant in the brain, eye, and central nervous system.

Astaxanthin is a more stable antioxidant than vitamin E because the radical is incorporated into its polyene chain as opposed to allowing the radical to float free. Astaxanthin also exhibits far greater anti-inflammatory protection than vitamin E where reactive oxygen is involved. The mechanism of action of this anti-inflammatory protection is unclear at present but may be related to singlet oxygen quenching, which would protect against active oxygen-induced membrane damage.

In addition to the scientific findings that astaxanthin is a powerful antioxidant and can serve as a potent scavenger of free radicals in a variety of tissue types, astaxanthin has been found to provide many essential biological functions. Astaxanthin provides protection against lipid-membrane peroxidation of essential polyunsaturated fatty acids and proteins, DNA damage and UV light effects, and it also plays an important role in immunological defense.¹

III. Levels and Conditions of Use

U.S. Nutraceuticals submits that astaxanthin extract is reasonably expected to be safe for use in dietary supplements at a level of up to five milligrams per day (5 mg/day). There are no other conditions of use.

IV. Reasonable Expectation of Safety for Use in Dietary Supplements

The safety of both astaxanthin and the *Haematococcus pluvialis* algae from which it is commonly derived have been studied extensively and is well documented both in past submissions to FDA and in the scientific literature. The safety of *Haematococcus pluvialis* algae is best summarized in technical bulletins prepared by Cyanotech Corporation and Aquasearch, Inc. (a.k.a. Mera Pharmaceuticals) to support the safety of their dietary new dietary ingredients derived from the algae. These bulletins are attached as Appendix H.

As for the safety of astaxanthin specifically, Roche Vitamins and Fine Chemicals, a division of Hoffman-La Roche, Inc., submitted a petition to FDA on August 11, 1987, for the approval of astaxanthin as a feed additive in salmonid fish. The safety studies contained in the Roche petition are summarized in section A. Additional safety studies on astaxanthin are summarized in section B. Furthermore, a number of New Dietary Ingredient Notifications regarding astaxanthin-related ingredients – each of which contains discussions of the safety of astaxanthin – have been submitted to FDA and the agency has found no objection to these petitions. Brief descriptions of these notifications are compiled in section C.

A. Roche Safety Studies

The Roche petition consists of seven volumes and is attached as Appendix I. The safety evaluation performed included studies on the acute toxicity, mutagenicity, teratogenicity, embryotoxicity, reproductive performance, and tolerance as astaxanthin. These studies, from which it was concluded that astaxanthin is safe reasonably expected to be safe at the doses tested, are summarized as follows.

1. Acute Toxicity

The acute toxicity of astaxanthin was tested in two studies. The first study involved the oral or intraperitoneal administration of astaxanthin to mice and rats at levels exceeding 8000 mg/kg body weight over a period of 10 days. The second study involved the oral

administration of astaxanthin at levels exceeding 2000 mg/kg body weight over a period of 10 days. There was no mortality and no symptoms of toxicity reported in either study.

2. Mutagenicity

The mutagenicity of astaxanthin was tested in two studies. In the Ames test, no mutations were induced in *Salmonella typhimurium* strains TA 1535, TA 1537, TA 1538, TA 98, and TA 100, with or without activation by rat liver homogenate fraction, at concentrations of astaxanthin ranging from 0.03 to 5.0 mg/plate. In the micronucleus test, astaxanthin was administered orally to mice in a 10% gelatin beadlet at doses of 500, 1000, and 2000 mg/kg body weight. No increase in micronuclei was observed, and it was concluded that astaxanthin did not induce chromosome breaks or mitotic disjunction *in vivo*.

3. Teratogenicity and Embryotoxicity

Astaxanthin was tested for teratogenic and embryotoxic effects in two studies involving rabbits and rats, respectively. In rabbits, astaxanthin was administered to pregnant females at doses of 0, 100, 200, and 400 mg/kg body weight/day from day 7 to 19 of gestation, and the animals were sacrificed at day 30 of gestation. The fetuses were removed by ovariectomy, tested for viability (24 hours), and then examined for macroscopic, skeletal, and visceral and soft tissue anomalies. All females tolerated astaxanthin well and no signs of maternal sensitivity or changes in body weight were observed, relative to controls. No dose-related effects were observed in reproductive and litter parameters, course and outcome of pregnancies, and malformations among the fetuses examined. Also, there was no evidence of teratogenic effect at the highest dose group.

In rats, astaxanthin was administered to pregnant females at doses of 0, 250, 500, and 1000 mg/kg body weight/day in gelatin beadlets on days 7 to 16 of gestation. No maternal deaths or signs of maternal toxicity were observed, with the exception of a dose-dependent reduction in weight gain during the treatment period. No embryotoxic or teratogenic effect was observed at any dose level, and there was no indication of functional abnormalities.

4. Reproductive Performance

Astaxanthin was tested for reproductive effects in a two-generation study in rats. Doses of 0, 25, 100, and 4000 mg/kg body weight/day were administered by oral gavage to groups of 32 male and female rats. The males were administered astaxanthin beginning 70 days prior to mating and continuing through sacrifice, while the females were administered astaxanthin from 14 days prior to mating and continuing through gestation until sacrifice or weaning. No substance-related mortality in any dosage group of either sex of the parental generation was observed. Body weight gain in all groups was comparable to controls. The percentage of males that mated as well as the ratio of mated to pregnant females and precoital time were comparable in all groups. The reproductive parameters of females sacrificed during gestation were within the normal limits.

In the first generation, litter parameters (i.e., pup weight gain, onset time of developmental landmarks, and learning and memory ability) were comparable in all dose groups. Neonatal mortality was within the limits of the biological range for all groups. Isolated anomalies were observed in all groups and were not considered dose-related. In the second generation, a higher rate of mortality or cannibalism, relative to the first generation, was observed across all groups, including controls. It was concluded that the doses of astaxanthin administered had no effect on the gametogenesis, mating, gestation, and lactation of rats.

5. Tolerance

Astaxanthin was tested for tolerance in two 13-week studies in rats and dogs, respectively. In rats, astaxanthin was administered in doses of 0, 310, 620, and 1340 mg/kg body weight/day via gelatin beadlets added to feed. No astaxanthin-related mortality was observed, and body weights in all groups were comparable. Animals fed astaxanthin exhibited reddish feces and, at autopsy, yellow adipose tissue pigmentation. Decreased organ weights were observed in experimental groups, although the histology was similar to the control group. A decrease in total serum protein levels and higher plasma cholesterol levels (within limits) were observed in the higher dose groups. Other slight abnormalities were judged to be potentially parasite related and, therefore, not associated with astaxanthin intake. It was concluded that astaxanthin was well

tolerated and that no apparent toxicity attributable to astaxanthin was observed.

In dogs, gelatin beadlets containing 0, 2.5, 5 and 10% astaxanthin were added to the feed, resulting in an average intake of 0, 41, 76, and 162 mg/kg body weight/day, respectively. Each experimental group consisted of three male and three female dogs. No adverse effects or evidence of systemic toxicity were observed in any of the dosage groups in terms of state of health, body weight development, behavior, hematological and clinical parameters, ophthalmoscopy, and autopsy and appearance of organs.

B. Additional Safety Studies

A number of other studies have been performed on astaxanthin or *Haematococcus* derivatives primarily composed of astaxanthin. These studies are summarized as follows.

1. Subacute Toxicity

Murillo performed a 30-day oral feeding study in four groups of male Wistar rats in which the animals were fed synthetic diets containing no carotenoids or 0.1 % of beta-carotene, canthaxanthin, or astaxanthin, respectively. While the group fed the beta-carotene had no change in plasma cholesterol levels, the groups fed the canthaxanthin and astaxanthin diets had a significant increase in plasma cholesterol levels. The increase was observed to consist mainly of an increase in the HDL fraction of the lipoprotein (i.e., the “good” cholesterol).³

2. Subchronic Toxicity

Ono, *et. al* performed a 13-week oral toxicity study on *Haematococcus* color, a feed additive consisting mainly of astaxanthin, in F344 rats. Four groups of rats, consisting of 10 male and 10 female animals each, were administered diets containing 0, 0.5, 1.5, and 5% of CRF-1 powder (corresponding to 0, 0.025, 0.075, and 0.25% *Haematococcus* color). No mortality was observed during the study, and there were no exposure-related changes in body weight gain or food consumption. Biochemical analyses showed a slight dose-related increase in serum cholesterol levels, but this was determined to not be an adverse effect. No treatment-related effects were observed in hematological parameters, organ

weights, and histopathological examinations. The authors concluded that the ingestion of *Haematococcus* color by rats over a period of 13 weeks did not cause any toxicological changes.⁴

3. Clinical Studies

The safety of astaxanthin consumption in humans has been established in a number of clinical studies.

In a recently published study by Spiller and Dewell, 35 healthy adults (age 35 to 69 years) participated in a randomized, double-blind, placebo-controlled trial over an eight week period. Members of the treatment group consumed one gelcap of *Haematococcus pluvialis* algal extract in safflower oil per meal (containing 2 mg of astaxanthin) for a total consumption of 6 mg of astaxanthin per day, while members of the control group consumed placebo gelcaps containing only safflower oil. A variety of blood chemistry parameters were measured at the beginning of the trial and after four and eight weeks of supplementation. There were small, statistically significant differences in serum calcium, total protein, and eosinophils, but these differences were determined to be of no clinical importance.⁵

A proprietary study performed by Mera Pharmaceuticals (aka Aquasearch) demonstrated that daily ingestion of either a low dose of 228 mg of *Haematococcus pluvialis* algal meal (3.85 mg of astaxanthin) or a high dose of 1,140 mg of *Haematococcus pluvialis* algal meal (19.25 mg of astaxanthin) over a period of 29 days did not result in any safety concerns. The study involved 33 volunteers (15 males and 18 females, age 28 to 62 years), and periodic blood and urine analyses and a detailed medical examination were performed during the study. No signs of toxicity were observed. (See study summary in technical bulletin prepared by Mera Pharmaceuticals in Appendix H.)

In addition, a study performed by Miki, et al. demonstrated that the ingestion of up to 14.4 mg/day of astaxanthin over a period of two weeks resulted in no ill effects. (See study summary in technical bulletin prepared by Mera Pharmaceuticals in Appendix H.)

C. Previous Astaxanthin-Related New Dietary Ingredient Notifications

U.S. Nutraceuticals notes that the safety-related data in the following New Dietary Ingredient Notifications for astaxanthin-related ingredients were filed in Docket No. 95S-0316 and not objected to by FDA:

- Neptune Technologies & Bioresources – premarket notification for Krill-based ingredients filed on May 15, 2002 (RPTs 131, 132, 133 and 145);
- Micro Gaia Inc. – premarket notification for astaxanthin extract of *Haematococcus pluvialis* algae filed on March 7, 2002 (RPT 119);
- Igene Biotechnology, Inc. – premarket notification for *Phaffia rhodozyma* filed on May 4, 2000 (RPT 74);
- Aquasearch, Inc. – premarket notification for *Haematococcus pluvialis* algae filed on February 22, 2000 (RPT 65); and
- Cyanotech Corp. – premarket notification for *Haematococcus pluvialis* algae filed on March 22, 1999 and May 25, 1999 (RPTs 45 and 50).

D. Intake Exposure Levels

Primarily due to its natural presence in salmonid fish and shellfish, astaxanthin has been consumed by humans at substantial levels throughout the world. Average levels of astaxanthin in wild Pacific salmon have been measured to be up to 14 mg/kg in Coho salmon and 40 mg/kg in Sockeye salmon.⁶ Also, astaxanthin is used world-wide to supplement fish feeds and is approved in the United States as a color additive for feed for salmonid fish under 21 CFR § 73.35 at levels up to 80 mg/kg of feed. Farm-raised Atlantic salmon are commonly fed feed containing 50 mg or more of astaxanthin per kg of feed for periods of up to two years. Such practices lead to levels of astaxanthin in the flesh of farmed Atlantic salmon of 4 to 10 mg/kg, which are comparable to slightly higher than the astaxanthin levels found in wild Atlantic salmon. Based on the levels of astaxanthin found in wild and farm-raised salmon, the daily consumption of a pre-cooked, 110-gram serving (approximately 4 ounces) of these fish would result in an intake of astaxanthin of 1.5 mg/day for Coho salmon, 4.4 mg/day for Sockeye salmon, and up to 1.1 mg/day for Atlantic salmon.

Humans have also consumed astaxanthin from a variety of sources in dietary supplements. Use of astaxanthin in dietary supplements are supported by New Dietary Ingredient Notifications accepted for filing by FDA in Docket No. 95S-0316. The following Notifications, along with the proposed daily intakes and sources of astaxanthin, have been filed with FDA:

- Aquasearch: 5 mg of astaxanthin per day from *Haematococcus pluvialis* algal meal with no apparent restrictions;
- Micro Gaia: up to 2 mg of astaxanthin per day from extract of *Haematococcus pluvialis* algae with a warning that pregnant and lactating women should consult a physician before use;
- Neptune Bioresources: unspecified levels of astaxanthin per day from up to 900 mg of Krill-derived products with restrictions on use by people with seafood allergies and those taking anticoagulants;
- Igene Biotechnology: 2 mg of astaxanthin per day from *Phaffia rhodozyma* with restrictions on use by children and chronic/long-term use;
- Cyantotech: 2 mg of astaxanthin per day from up to 600 mg of *Haematococcus pluvialis* algae powder with no apparent restrictions.

E. Summary

All of the information summarized above demonstrates that astaxanthin is well tolerated and can be safely consumed at levels up to and exceeding 5 mg/day. The toxicity studies described herein were performed on a variety on animal species, for extended periods, and at very large dose levels. The clinical trials demonstrate that the intake of up to 19.25 mg of astaxanthin per day over a one-month period and 6 mg of astaxanthin per day over an eight-week period are well tolerated by humans. Collectively, these studies demonstrate that the toxicity of astaxanthin is extremely low or nonexistent. Furthermore, the safety of *Haematococcus pluvialis* algae, a common source of astaxanthin, is well established. And the safety of astaxanthin from a variety of sources, including dietary supplements, has been well documented.

V. Conclusion

As supported by the data presented in this New Dietary Ingredient Notification, U.S. Nutraceuticals submits that the consumption of up to 5 mg per day of astaxanthin from ZANTHIN® Extract Astaxanthin Complex – 10% Standardized, an astaxanthin-rich carotenoid oleoresin derived from *Haematococcus pluvialis* algae is safe. U.S. Nutraceuticals will begin marketing its ZANTHIN® Extract Astaxanthin Complex – 10% Standardized product 75 days after acknowledgement of FDA's receipt of this notification unless otherwise instructed by the agency.

¹ Wiener, H., Drory, A., and Line, L. (2003) Astaxanthin from the microalga *Haematococcus* – a superb natural antioxidant for human health. Innovations in Food Tech., (Nov.): 22-26.

² Miki, W. (1991) Biological functions and activities of animal carotenoids. Pure & Applied Chem., 63(1): 141-146.

³ Murillo, E. (1992) Hypercholesterolemic effect of canthaxanthin and astaxanthin in rats. Arch. Latinoam. Nutr., 42(4): 409-413 (abstract only).

⁴ Ono, A., Sekita, K., Saitoh, M., Umemura, T., Ogawa, Y., Furuya, T., Kaneko, T., and Inoue, T. (1991) A 13-week subchronic oral toxicity study of *Haematococcus color* in F344 rats. Kokuritsu Iyakuin Shokuhin Eisei Kenkyusho Hokoku, 117: 91-8 (abstract only).

⁵ Spiller, G.A., and Dewell, A. (2003) Safety of an astaxanthin-rich *Haematococcus pluvialis* algal extract: a randomized clinical trial. J. Medicinal Food, 9(1): 51-56.

⁶ Turujman, S.A., Warner, W.G., Wei, R.R., and Albert, R.H. (1991) Rapid liquid chromatographic method to distinguish wild salmon from aquacultured salmon fed synthetic astaxanthin. J. AOAC Int., 80(3): 622-632.

* The cited documents are compiled in Appendix J.