

**New Dietary Ingredient Notification for
Astaxanthin Extracted from *Haematococcus* Algae**

(Volume 1 of 2)

**Fuji Chemical Industry Co., Ltd.
March 5, 2004**

ULLMAN, SHAPIRO & ULLMAN, LLP

COUNSELORS AT LAW

299 BROADWAY, SUITE 1700

NEW YORK, NY 10007

TEL. (212) 571-0068

FAX. (212) 571-9424

www.usulaw.com

usu@usulaw.com

ROBERT ULLMAN
STEVEN SHAPIRO*
MARC S ULLMAN

SETH A. FLAUM*^o
VANESSA RIVIERE*

TRADEMARK COUNSEL
CHARLES H. KNULL^o

BUSINESS & TECHNOLOGY COUNSEL
IRA R. HECHT*^o†

OF COUNSEL
IRVING L. WIESEN

* ADMITTED IN NY & NJ
^o ADMITTED IN NY & DC
^o ADMITTED IN MD & DC
^o ADMITTED IN FL
† CPA

WASHINGTON AFFILIATE
JAMES M. JOHNSTONE
1776 K STREET, NW
WASHINGTON, DC 20006

LONDON AFFILIATES
WEDLAKE BELL
16 BEDFORD STREET
COVENT GARDEN
LONDON WC2E 9HF
ENGLAND

E. U. CORRESPONDENT
LAFILL VAN CROMBRUGGHE
& PARTNERS
VOSSENDREEF 6 BUS 1
B-1180 BRUSSELS,
BELGIUM

March 5, 2004

Via Federal Express

Division of Standards and Labeling Regulations
Office of Nutritional Products, Labeling, and Dietary Supplements (HFS-820)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835

MAR 15 2004

LB/FDA

**Re: New Dietary Ingredient Notification for Astaxanthin Extracted from
Haematococcus Algae**

Dear Sir/Madame:

Pursuant to 21 CFR § 190.6, please be advised that Fuji Chemical Industry Co., Ltd.¹ ("Fuji Group"), the parent company of BioReal, Inc.² (formerly Micro Gaia, Inc.), AstaReal AB³ and FCI Health Science, Inc.⁴ is hereby providing you with notification of its intent to market the dietary ingredient, Astaxanthin extracted from *Haematococcus* algae, at a dose of up to 12 mg per day. In January 2002, Micro Gaia notified FDA of its intent to market this ingredient with a recommended daily dose of 1-2 mg per day. (Attached) On May 21, 2003, FDA acknowledged receipt of Micro Gaia's notification and, after careful review, concluded that "Astaxanthin extracted from the *Haematococcus pluvialis* algae and consumed at the recommended dose of 1-2 mg per day will reasonably be expected to be safe." (Attached)

Based on its previous notification to FDA and upon the following, Fuji Group respectfully submits that there are no safety issues relating to the intended marketing of Astaxanthin extracted from *Haematococcus* algae at daily doses of 2 - 12 mg per day:

¹ The company's facilities are located at: 55 Yokohoonji, Kamiichi, Nakaniikawa, Toyama, 930-0397, Japan.

² The company's facilities are located at: 1777 Pi'ilani Highway, Kihei, Hawaii 96753.

³ The company's facilities are located at: Idrottsvägen 4, Gustavsberg, Sweden, SE-134 40.

⁴ The company's facilities are located at: 560 Fellowship Road, Suite 316, Mount Laurel, New Jersey 08054.

7 7692

ULLMAN, SHAPIRO & ULLMAN, LLP

Division of Standards and Labeling Regulations

Office of Nutritional Products, Labeling, and Dietary Supplements (HFS-820)

Center for Food Safety and Applied Nutrition

March 5, 2004

Page 2

1. Fuji Group is a pharmaceutical ingredient supplier and has been in operation since 1946. It is one of the leading custom manufacturers of bulk pharmaceuticals and chemicals in Japan, the United States and the European Union.
2. AstaReal AB, formerly known as AstaCarotene AB, was the first to successfully develop the cultivation process and market commercial products derived from *Haematococcus pluvialis*. Since 1995, AstaReal AB has been marketing its own cultivated Astaxanthin dietary supplement. It is currently sold in Scandinavia and the United Kingdom.
3. Astaxanthin extract is derived from *Haematococcus* algae and is grown at two Fuji locations: i) at the BioReal facility in Maui, Hawaii and ii) at the AstaReal facility in Gustavberg, Sweden.
4. As described in Micro Gaia's 2002 notification, the Maui facility uses proprietary Bio-Dome technology designed to ensure that the algae is grown under sterile conditions, free from pollutants and other environmental toxins. The facility has been inspected by the State of Hawaii and has been found to be in compliance with all applicable rules and regulations. The algae grown at the Maui facility will be extracted at the Fuji facility in Toyama, Japan and at the Phasex Corporation, located in Lawrence, MA.⁵
5. Fuji Group's Toyama facility operates in compliance with all Current Good Manufacturing Practices applicable to companies whose pharmaceutical products are imported into the United States.
6. The AstaReal facility located in Sweden uses stainless steel bioreactor tanks also designed to ensure sterile conditions. The facility has been inspected by the Swedish Health Authority and is in compliance with all rules and regulations. Algae grown at this facility will also be extracted at the Fuji facility in Toyama and at the Phasex Corporation.
7. Astaxanthin produced in Gustavberg is registered and approved for sale as a dietary supplement in the E.U. More than 500,000 retail units have been sold since its launch. This represents nearly 30 million doses at a recommended daily dose of 4 mg. To date, no reports of adverse effects have been reported.
8. Fuji intends to market Astaxanthin extract under the trade names AstaREAL™ and AstaCarox®.
9. AstaREAL™ will initially be marketed in tablet and softgel capsule form. Each tablet/softgel will contain 5-6 mg of Astaxanthin extract. The recommended dose of AstaREAL will be 2-12 mg per day. The attached report of Harry G. Preuss, M.D.⁶

⁵ Phasex Corporation is located at 360 Merrimack Street Lawrence, MA 01843.

⁶ Professor of Physiology, Medicine and Pathology, Georgetown University Medical Center, Washington, D.C.

- ("Preuss Report") supports the safety of Astaxanthin at the recommended daily dose of 2 - 12 mg.
10. Astaxanthin has been approved for use in Salmonid feeds for a number of years. It is also found in the natural human food supply, especially in salmon, rainbow trout, lobster, shrimp and other fish species. Based on a recent survey of salmonid fish, the average concentration of Astaxanthin is 13.2 mg/kg. The Astaxanthin concentration ranges from 1-8 mg/kg in Wild chum salmon, to 30-58 mg/kg in Wild Sockeye salmon. Based on the average concentration of all salmon listed, the intake of Astaxanthin from a typical salmon consumption at one sitting would be 3.3 mg - 6.6 mg.
 11. *Haematococcus* algae is the most prevalent commercial source of Astaxanthin and is believed to accumulate the highest levels of Astaxanthin in nature (1,000 to 3,000 higher than salmon). The Preuss Report indicates that no toxicity associated with *Haematococcus* has ever been reported in scientific literature.
 12. The general composition of *Haematococcus* algae consists of common carotenoids, fatty acids, proteins, carbohydrates and minerals. The Preuss Report concludes that Astaxanthin is as safe as other carotenoids and has been found to have similar absorption characteristics as other carotenoids.
 13. The data submitted by Roche Vitamins in support of the Color Additive Petition 7C0211 for Astaxanthin includes numerous toxicity studies in animals supporting the safety and tolerance of Astaxanthin at doses ranging from 500 mg to 8,000 mg/kg body weight. Astaxanthin is approved for use in Salmonid feed at a maximum level of 80 mg/kg pursuant to 21 CFR §73.35. Materials from the Roche Petition have already been submitted to FDA as part of Micro Gaia's first notification for Astaxanthin.
 14. Numerous toxicity studies in animals indicate that extremely high doses of Astaxanthin are safe in animals. In one study, it was concluded that *Haematococcus pluvialis* shows no evidence of mutagenic/clastogenic activity after administration of 2000 mg/kg. Attempts to find an LD50 for *Haematococcus pluvialis* have failed and doses as high as 8g/kg administered for 10 days caused no evident toxicity.
 15. Salmon is the main source of protein among native Alaskans. Daily Astaxanthin intake for Eskimos has been estimated at 8mg based on a daily consumption of 200g portions of wild Sockeye salmon. This high consumption of Astaxanthin has been associated with a low rate of ischemic heart disease mortality.
 16. The review of 11 human studies shows no adverse health events from consumption of Astaxanthin for extended periods of time, even at doses of 4 mg/day for 6 months and 40 mg/day for 8 weeks. Human safety studies indicate that daily doses of Astaxanthin up to 12 mg are safe for several months.

ULLMAN, SHAPIRO & ULLMAN, LLP

Division of Standards and Labeling Regulations

Office of Nutritional Products, Labeling, and Dietary Supplements (HFS-820)

Center for Food Safety and Applied Nutrition

March 5, 2004

Page 4

17. Although one study showed a physiological effect on oxidized LDL, no adverse effects were reported at doses of Astaxanthin ranging from 3.6 mg/day to 14.4 mg/day.

18. The Preuss Report concludes that "the present evidence points to complete safety in the use of astaxanthin" and that "daily doses of 2-12 mg/day would be safe for many months."

In light of the above, Fuji Group hereby incorporates by reference FDA's acceptance of safety related data in connection with docket number 95S-0316 by the following companies:

- Micro Gaia, Inc. March 1, 2002
- Igene Biotechnology, Inc May 4, 2000
- Cyanotech Corp. May 25, 1999
- Aquasearch, Inc. December 16, 1999
- Cyanotech Corp. March 22, 1999

Based on the foregoing, we believe that FDA should accept this filing on behalf of Fuji Group as providing sufficient evidence that Astaxanthin extracted from *Haematococcus* algae, when used under the conditions recommended in the product labeling, can reasonably be expected to be safe for human consumption. In support of this, we have also included testing data which reflects the purity of Fuji Group's product as manufactured.

If you require any additional information, please direct all correspondence to the undersigned.

Very truly yours,

ULLMAN, SHAPIRO & ULLMAN, LLP



Marc S. Ullman
Vanessa Riviere

Encls.
(Volumes 1 & 2)

To Marc Ullman
Ullman, Shapiro, & Ullman
New York, NY.

From: Harry G. Preuss MD, MACN, CNS
Professor of Physiology, Medicine, and Pathology
Georgetown University Medical Center
Washington, D.C.

Re: Safety of Astaxanthin at doses up to 12.0 mg/day

Date: January 15, 2004

Dear Mark,

At your request, I have examined the literature pertaining to the safety of astaxanthin in order to determine the safety of daily doses in the range >2.0 and up to 12.0 mg/day. The report below was derived from material provided by Fuji Chemical of Japan and AstaCarotene AB of Gustavsberg, Sweden, materials obtained from the Internet, from PubMed (NIH) and from various companies involved in the manufacture of the antioxidant.

I have divided the report into three sections: evidence for safety from dietary findings, animal toxicity studies and human toxicity studies.

EVIDENCE FOR SAFETY FROM DIETARY FINDINGS

Astaxanthin is a red pigment that provides color to many living organisms [1,2]. Natural astaxanthin gives salmon, lobster, and shrimp their red color. Astaxanthin is prevalent in human foods, especially salmon and rainbow trout and also occurs in lobsters and shrimps, in fish eggs, and in other fish species [3]. Accordingly, the pigment is found in many human foods and consumed by many over long periods of times, e.g., years. In commercial fish and crustacean farms, astaxanthin is commonly added to feeds to make up for a lack of natural dietary sources [4,5]. Indeed, it was found to be necessary for the growth and survival of marine life [4].

A recent survey of salmonid fish found the following concentrations of astaxanthin [6]:

SPECIES	ASTAXANTHIN RANGE	ASTAXANTHIN MEAN
Wild sockeye salmon	30-58 mg/kg	40.4 mg/kg
Wild Coho salmon	9-28 mg/kg	13.8 mg/kg
Wild pink salmon	3-7 mg/kg	5.4 mg/kg
Wild chum salmon	1-8 mg/kg	5.6 mg/kg
Wild Chinook king salmon	1-22 mg/kg	8.9 mg/kg
Wild Atlantic salmon	5-7 mg/kg	5.3 mg/kg

Average of all species = 13.2 mg/kg

Based on these concentrations, a typical salmon consumption at one sitting of 0.25-5.0 kg would result in a low intake of 1.33-2.65 mg of astaxanthin from the Atlantic salmon to a high intake of 10.1-20.2 mg from the sockeye salmon. Based on the average concentration of all salmon listed, the intake of astaxanthin from salmon would be 3.3-6.6 mg. The requested dose (2.0 to 12.0 mg/day) of astaxanthin for the dietary supplement produced by Fuji Chemical of Japan reasonably fits into this range.

A ubiquitous algae is the most prevalent commercial source of astaxanthin. *Haematococcus pluvialis* algae occur worldwide and are believed to be the organism that can accumulate the highest levels of astaxanthin in nature. This organism accumulates 1,000 to 3,000 higher levels than salmon. Of importance, no toxicity associated with *Haematococcus* has ever been reported in the literature [7]. The general composition of *Haematococcus* algae consists of common carotenoids, fatty acids, proteins, carbohydrates and minerals. *Haematococcus* algae meal has been approved in Japan as a natural food color and as a pigment in fish feeds.

The structure of astaxanthin is similar to that of other carotenoid pigments like beta carotene (carrots), lycopene (tomatoes) and lutein (spinach) [8]; however

astaxanthin is a more powerful antioxidant than either beta carotene or vitamin E [9,10]. Like other carotenoids, astaxanthin cannot be synthesized in animals and must be provided in the diet. Because of its strong antioxidant capabilities, astaxanthin has been postulated to have some benefits in cardiovascular diseases like atherosclerosis and strokes, macular degeneration, cancer and various neurodegenerative diseases such as Alzheimer's and Parkinson's diseases. Much interest has been focused on the latter two diseases, because astaxanthin can pass the "blood brain barrier", meaning it can deliver antioxidant benefit directly to the brain and central nervous system. Over the last few years, inflammation has become a hot topic. A study conducted at Kochi Medical School in Japan found artificially induced inflammation of rat paws was significantly inhibited by astaxanthin [11].

SUMMARY

Astaxanthin is a carotenoid and should be as safe as other carotenoids. In addition, astaxanthin has been found safe as an animal supplement and is already in the human food chain.

ASTAXANTHIN TOXICITY-RELATED STUDIES IN ANIMALS

Astaxanthin has been approved as feed for salmonid fish for a number of years. The original petition for use as a feed was filed by Roche Vitamins and is in 7 volumes [12]. Acute toxicity studies in animals were carried out in rats and mice over ten days and challenges up to 8000 mg/kg body weight showed no mortality or toxicity. When mutagenicity was tested, no mutations were induced in *Salmonella typhimurium* strain using concentration of 0.03-0.05 mg/plate in the Ames test whether rat liver homogenates were activated or not. In another test of mutagenicity using the Micronucleus test, no chromosomal breaks nor mitotic dysjunctions were found *in vivo* with doses of 500, 1000, and 2000 mg/kg of body weight. Concerning teratology and embryotoxicity studies, doses of 100, 200, and 400 mg/kg/day were given to pregnant rabbits for a 12 day period. At 30 days of gestation, the examination of fetuses showed no malformations. The mothers all tolerated the astaxanthin well. In rats given astaxanthin, no embryotoxic or teratogenic effects of astaxanthin were observed. Reproductive studies on rats and dogs revealed no perturbations from the high doses used. Thirteen-week tolerance tests on rats and dogs revealed no toxic effects.

A series of *in vitro* safety tests for *Haematococcus pluvialis* were carried out by Scantox, Lille Skensved, Denmark in 1998 [13-15]. These included the mouse micronucleus test, the Ames test and the *in vitro* mammalian cell gene mutation test performed with mouse lymphoma cells. Based on the Mouse Micronucleus Test [13], it was concluded that *Haematococcus pluvialis* showed no evidence of mutagenic/clastogenic activity after administration by oral gavage of 2000 mg/kg. The Ames Test also found no evidence of mutagenicity [14], and *Haematococcus pluvialis* was not mutagenic in the *in vitro* mammalian cell mutation test performed with mouse lymphoma cells in the presence and absence of S-9 mix [15].

Three different lots of *Haematococcus* algae were administered by gavage to Charles River CD rats (12). 5,000 mg/kg were administered in a 0.5% aqueous methycellulose solution. In a thirteen day study on both male and female rats, no visible abnormalities were noted and the rats continued to gain weight during the entire study. In another study, doses of 10,417-18,000 mg/Kg given by oral gavage did

not reveal any toxicity, even when organs were examined postmortem. Therefore, the LD 50 was judged to exceed 18,000 mg/kg [12].

Studies performed at the Instituto Di Ricerche Biomedichein Italy attempted to establish an LD 50 for *Haematococcus pluvialis* [16]. The test article was given in suspension form at doses as high as 12g/kg. No rats died during the study and it judged that the LD 50 was higher than 12g/kg. No clinical signs or behavioral alterations were noted during the observation period in any animal and body weight was unaffected by treatment. At autopsy, no gross changes were seen. In another study from the Instituto Di Ricerche Biomedichein Italy, *Haematococcus pluvialis*, unicellular green algae was administered by the oral route as a suspension in an intralipid solution once a day for 14 consecutive days at the maximum administrable dose of 6g/kg/day [17]. Each experimental group was composed of 6 male and 6 female rats. No treatment-related deaths occurred and routine clinical observations and laboratory investigations did not show any adverse changes in the test animals of either sex.

A 13-week oral repeated dose toxicity study of *haematococcus color*, a food additive mainly composed of astaxanthin, was conducted in male and female F344 rats (18). Rats were randomly divided into 4 groups each consisting of 10 males and 10 females and given a powder diet containing 0, 0.025, 0.075, and 0.25% *haematococcus*, i.e., 0, 0.5, 1.5, and 5% as the product. None of the animals died during the administration period. There were no exposure-related changes in body weight gain or food consumption. Serum biochemical examinations showed a dose-related increase in cholesterol, but the differences were slight and not defined as an adverse effect. No effects related to treatment were noted in the hematological examinations and organ weights, and no abnormalities that could be ascribed to exposure to *haematococcus color* were observed in histopathological examinations. The authors concluded that ingestion of *haematococcus color* in the diet for 13 weeks did not cause any toxicological changes in F344 rats.

In another 13 week study [19], algae meal HPP was given to 40 male and 40 female rats of the Cri:WI(Glx/BRL/Han)BR strain from Charles River UK Limited in doses sufficient to give dietary concentrations of 1, 5, and 20% to groups of 10 rats. The trans-astaxanthin component of the test material was quantifiable in the plasma of all high dose animals, demonstrating exposure. No quantifiable levels were found in control rats. Orange fur staining and orange colored feces were recorded for all animals that received the algae. No deaths occurred. Body weights were unaffected by treatment and food consumption was essentially similar to that of the controls for most treated groups. However, high dose females ate marginally less food than their controls overall. There were no inter-group differences in the hematological composition or clotting potential of the blood that could confidently be attributed to treatment. The high dose males showed a slight increase in alkaline phosphatase activity and the intermediate and high dose rats showed a slight increase in circulating cholesterol. The authors concluded that the administration of the test article at dietary levels up to 200,000 ppm for 13 weeks was well tolerated, and was without any adverse signs of toxicity – being associated with only minor histological changes in the kidneys. They considered that a dose level of 200,000 ppm represented the No-Observed-Adverse-Effect level (NOAEL) under the conditions of the study.

Three groups of male Wistar rats (130-140g) were fed 30 days with synthetic diets containing 0.1% of beta carotene, canthaxanthin, and astaxanthin (20). Another group was fed with a synthetic diet without carotenoids. The results showed that the beta carotene did not induce change in plasma cholesterol, but canthaxanthin and astaxanthin induced a significant increase in cholesterol concentration. However, the increase was due mainly to HDL, the so-called good cholesterol.

Fish tissues from a Haematococcus algae feeding to rainbow trout were analyzed for toxic effects and neoplasia [1]. All tissues examined were normal in appearance with no indication of disease, toxicity, or neoplasia. All fish examined were in excellent nutritional status with abundant body fat. Gross findings indicate that no adverse effects on health were observed from Haematococcus algae meal as the dietary source of astaxanthin.

In one report, the physiological effectiveness and safety of astaxanthin and beta-carotene were studied by feeding them to rats [21]. Rats took two different doses of astaxanthin (80 and 400 mg/kg of diet). With high doses of astaxanthin or with beta-carotene, rats grew normally during the 41 days of study. No toxicities were observed on body weight, hair appearance, nosebleeds, organ weight or liver enzymes. No harmful effects were seen on pregnancies or deliveries of rats.

In a bacterial reverse mutation test of astaxanthin performed by the Nippon Medical Research Institute Co, the mutagenicity of astaxanthin was examined using histidine requiring tester strains of TA98, TA 100, TA 1535, and TA 1537 (Salmonella typhimurium) [22]. From the findings, it was concluded that astaxanthin had no mutagenicity under the experimental conditions examined. A single oral dose toxicity study of astaxanthin in rats was carried out by the same group [23]. No deaths were seen and the rats grew normally after challenged with a dose of 2,000 mg/kg. The investigators concluded that the lethal dose was above 2,000 mg/kg in males and females under the conditions of the study.

SUMMARY

Animal studies, where extremely high doses of astaxanthin can be used, show a wide range of safety in the use of this antioxidant.

ASTAXANTHIN TOXICITY-RELATED STUDIES IN HUMANS

Interestingly, salmon is the main source of protein among native Alaskans and this high consumption has been associated with their low rate of ischemic heart disease mortality (<1/3 of Caucasians), i.e., the high consumption is associated with health rather than toxicity [24]. Based on values for salmon flesh, astaxanthin intake values for Eskimos with a daily consumption of 200 g portions of wild Sockeye salmon with 40 ppm astaxanthin would lead to a daily ingestion of 8 mg. As a further comparison, intake of a 5 mg supplement of astaxanthin corresponds to eating 500 g per day of rainbow trout or Atlantic salmon.

Astaxanthin was administered daily over 2 weeks to 5 subjects at 3.6 mg/day, to 5 subjects at 7.2 mg/day and to 3 subjects at 14.4 mg/day. A physiological effect on oxidized LDL was noted, but no adverse effects were reported at any dose (25).

Lignell (26) followed 40 healthy volunteers over 6 months: one half received 1 capsule containing 4 mg astaxanthin (algae meal) and the other one half received a placebo. No significant differences were observed between the two groups with the exception of the strength endurance tests. These improved on the antioxidant.

Thirty-three healthy adult volunteers were given astaxanthin obtained from *Haematococcus pluvialis* dry algae meal [27]. Each subject consumed daily over 29 days either a low dose (3.85 mg) or a relatively high dose (19.25 mg) astaxanthin. No adverse reactions were found after examining weight, skin coloration, general appearance, blood pressure, near and distant vision, color vision, depth perception, general eye condition, ears and nose, mouth, throat and teeth, chest and lungs, reflexes, blood analyses and urinalyses.

In a human study performed on 3 middle-aged males, a single dose of 100 mg astaxanthin had no negative effects and was found to have an absorption pattern similar to other carotenoids [28]. Because of similar absorption characteristics with other carotenoids, toxicity studies on other carotenoids may be important. Epidemiological studies in Northern Europe have found daily ingestion of carotenoids ranging from 2.9-7.6 mg per day [29-31]. The Alliance for Aging Research, an advocacy group, has recommended 10-30 mg beta-carotene per day for optimal health, and doses of 20-180 mg beta-carotene for many years have been used to treat erythropoietic protoporphyria without evidence of toxicity and without development of abnormally-elevated blood vitamin A levels [32]. Since astaxanthin has no pro vitamin A activity [33,34], it represents no risk for hyper-vitaminosis A.

In a recent study, Spiller and Dewell [35] conducted a human safety study on 35 healthy adults age 35-69 years. In this randomized, double-blind, placebo-controlled trial of 8 weeks duration, the subjects received a *Haematococcus pluvialis* algal extract with high levels of astaxanthin, i.e., they received 6 mg astaxanthin daily. They reported no significant clinical differences in hematology and blood chemistries. "These results reveal that 6 mg of astaxanthin per day from a *H. pluvialis* extract can be safely consumed by healthy adults."

A study carried out by the Sports and Health Science Department of Juntendo University in Japan examined visual acuity and muscular fatigue with and without astaxanthin [36]. The astaxanthin was provided by Fuji Chemical and given in doses of 6 mg/day for 4 weeks. A total of 34 subjects were examined, half on the astaxanthin. In addition to the four week of astaxanthin taking, the subjects were examined 4-5 weeks after going off astaxanthin. No signs of toxicity were found. Those receiving astaxanthin had improved deep vision and critical flicker fusion and lower lactic acid concentrations after intense aerobic activity.

Astaxanthin extract from *H. pluvialis* obtained from Fuji Chemical was given at a dose of 5 mg/day for 4 weeks to 13 test subjects [37]. No systemic adverse effects were noted in any subject. In the astaxanthin-supplemented group 7/13 had reduced

eyestrain as opposed to 1/13 of the placebo group who showed reduced eyestrain. No systemic adverse reactions were noted.

In a double blinded and randomized trial (n=25) divided into four groups supplemented with 2,4,8, and 12 mg per day for four weeks, blood chemistries, electrolytes, markers of liver and renal function and muscle breakdown, and hematological measurements were within normal limits at the end of study [38]. Only the A/G ratio showed an increase ($p<0.05$) in 4 mg and 12 mg groups in a non-dose dependent manner. The raised A/G results were still in the normal range.

Comhaire et al [39] examined 20 cases of sub fertile males over three months. 16 mg per day of astaxanthin was administered orally without the development of any adverse effects. The sperm count increased with the supplementation. Reactive oxygen species (ROS) in semen decreased significantly ($p<.05$) in the active group and remained unchanged in the placebo group. The pregnancy rate increased in the couples containing the active males.

Nagata at the Toyama International Research Center in Japan examined the effects of astaxanthin extract from *H pluvialis* on 20 subjects. In this double blind, randomized, cross-over study, the subjects received 5 mg/day of astaxanthin for 4 weeks. While the subjects were reported to improve resistance to fatigue on the supplement, there were no toxic adverse reaction noted [40].

Based on previous successful animal studies [41], a clinical study examining the ability of astaxanthin to improve symptoms of patients harboring *H pylori* was carried out [42]. 40 mg per day (5x8 mg) over 3 weeks was given to 10 patients. The entire study ran for 8 weeks. There were no treatment related hematological or clinical abnormalities. Significant improvements to symptom relief were reported.

SUMMARY

There have been no reports of serious adverse effects associated with astaxanthin administration to animals [16,18,19,23]. Attempts to find an LD 50 have failed to find a reasonable dose that will affect an animal adversely. Even a horrendous dose of 8 grams of astaxanthin per kilogram administered for 10 days caused no evident toxicity [43].

Although the studies are limited, human trials to date show no serious adverse events from consumption of astaxanthin. The attached table summarizes 11 human studies that showed no evidence of toxicity – even in those receiving 4 mg/day for 6 months and 40 mg/day for 8 weeks. The evidence suggests strongly that daily doses of 2-12 mg would be safe for many months.

In conclusion, the present evidence points to complete safety in the use of astaxanthin. Astaxanthin deserves no more concern when used properly than the use of other carotenoids such as those that do not have pro vitamin A activity [44].

BIBLIOGRAPHY

1. Torrissen OJ, Hardy RW, Shearer KD: Pigmentation of salmonids -- carotenoid deposition and metabolism. *CRC Crit Rev Aquat Sci* 1:209-225, 2000.
2. Naguib Y: Pioneering ataxanthin. *Nutrition Science News* February 2001, pp 1-6.
3. Aquasearch, Inc: Technical report TR.2102.001, 1999.
4. Torrissen OJ, Christianson R: Requirement for carotenoids in fish diets. *J Appl Ichthyol* 11:225-230, 1995.
5. Roche Laboratories: Carophyll Pink. <http://www.roche.com>.
6. Turujman SA, Wamer WG, Wei RR, Albert RH: Rapid liquid chromatographic method to distinguish wild salmon from aquacultured salmon fed synthetic astaxanthin. *J AOAC Int.* 80:622-632, 1997.
7. Technical Bulletin #060, Cyanotech Corporation: A technical review of *Haematococcus Algae*. 1999.
8. Terao J: Antioxidant activity of beta-carotene-related carotenoids in solution. *Lipids* 24:659-661, 1989.
9. Miki W: Biological functions and activities of animal carotenoids. *Pure Appl Chem* 63:141-146, 1991.
10. Iwamoto T, Hosada K, Hirano R, et al: Inhibition of low density lipoprotein oxidation by astaxanthin. *J Athero Thrombo.* 7:216-222, 2000.
11. Kurashige M, Okimasu E, Inoue M, Utsumi: Inhibition of oxidative injury of biological membranes by astaxanthin. *Physiol Chem Phys Med NMR* 22:27-28, 1990.
12. Roche Vitamins and Fine Chemicals: Hoffman La Roche, Inc. Astaxanthin as a pigmenter in salmon feed. CAP 7CO211.
13. Edwards CN: *Haematococcus pluvialis* mouse micronucleus test. Scantox Test Report Lab#26832, pp 1-14, April 1998.
14. Edwards CN: *Haematococcus pluvialis* Ames Test "Treat and Plate Test".. Scantox Test Report Lab#28708,, pp 1-20, June 1998.
15. Edwards CN: *Haematococcus pluvialis* In vitro mammalian cell gene mutation test performed with mouse lymphoma cells (L5178Y).. Scantox Test Report Lab#28709 pp 1-15, April 1998.
16. Tos EG: Acute toxicity study in rats treated by oral route. *Haematococcus pluvialis* unicellular green algae. *Instituto Ricerche Biomediceh.* RBM #950053. pp 1-21, Sept 1995.
17. Yu P, Maraschin R: 14 -day oral toxicity study in rats. *Haematococcus pluvialis*, unicellular green algae. RBM #9505101. . *Instituto Ricerche Biomediceh.* pp 1-31, Sept 1995.
18. Ono A, Sekita K, Saitoh M, Umenmukra T, Ogawa Y, Furuya T, Kaneko T, Inoue T: A 13 week subchronic oral toxicity study of *haematococcus color* in F344 rats. *Kokuritsu Iyakuin Shokuhin Eisei Kenkyushjo Hokoku* 117:91-98, 1999.
19. Stewart JS: HPP: 13 week oral (dietary administration) toxicity study in the rat. (Final ReportCovance Study Number 1840/002. pp 1-248, February 2001.
20. Murillo E: Hypercholesterolemic effect of canthaxanthin and astaxanthin in rats. *Arch Latinoam Nutr* 42: 409-413, 1992.
21. Yoshiyuki N, Yoshiharu M, Ichimura M: Physiological and biochemical effects of carotenoids (b-carotene and astaxanthin) on rats. *Bull Natl Health Sci* 2519-25, 1997.
22. Nippon Experimental Medical Research Institute Co, Ltd: Bacterial reverse mutation test of astaxanthin Project # H-02091, July 2002.

23. Nippon Experimental Medical Research Institute Co, Ltd: A single oral dose toxicity study of astaxanthin in rats Project # H-02227, December 2002.
24. <http://www.astafactor.com/techreports/tr3005-001.htm>. June 2003.
25. Miki WW, Hosoda K, Kondo K, Itakura H: Astaxanthin-containing drink. Patent application number 10155459. Japanese Patent Office. Publication date 16 June 1998.
26. Lignell A: Medicament for improvement of duration of muscle function or treatment of muscle disorder or diseases. Patent Cooperation Treaty application #9911251. AstaCarotene AB, Sweden.
27. Mera Pharmaceuticals, Inc.: Technical Report TR.3005.001, Haematococcus pluvialis and astaxanthin safety for human consumption, 1999.
28. Osterlie MB, Bjerkeng B, Liaaen-Jensen S: Pigments in food technology. Proc 1st Int Congr PFT, March 24-26, Sevilla Spain, 1999.
29. Golbohm RA, Brants HA, Hussoff KF, van den Brandt PA: The contribution of various foods to intake of Vitamin A and carotenoids in the Netherlands. Int J Vitam Nutr Res 68:373-383, 1998.
30. Heinonen M: Food groups as sources of retinoids, carotenoids and Vitamin A in Finland. Internat J Vit Res 61:3-9, 1991.
31. Faure H, Fayol V, Galabert C, Grolier P, Moel GL, Stephens J, Nabet F, Carotenoids: 2. Diseases and supplementation studies. Ann Biol Clin (Paris) 57:273-282, 1999.
32. VERIS: Carotenoids - Fact Book. Ed. VERIS, La Grange, IL, USA, 32pp. 1996.
33. Jyonouchi H, Sun S, Gross M: Effect of carotenoids on in vitro immunoglobulin production by human peripheral blood mononuclear cells: Astaxanthin a carotenoid without vitamin A activity, enhances in vitro immunoglobulin production in response to a T-dependent stimulant and antigen. Nutr Cancer 23:171-183, 1995.
34. Jyonouchi H, Sun S, Tomita Y, Gross M: Astaxanthin, a carotenoid without vitamin A activity, augments antibody responses in cultures including T-helper cell clones and sub-optimal doses of antigen. J Nutr 124:2483-2492, 1995.
35. Spiller GA, Dewell A: Safety of an astaxanthin-rich Haematococcus pluvialis algal extract: A randomized clinical trial. J Med Food 6:51-56, 2003.
36. Keisuke S, Yoshigi H, Aoki K, Koikawa N, Azumane A, Kaneko K, Yamaguchi M: Sports performance benefits from taking natural astaxanthin. Rinshou Iyaku 18:73-88, 2002.
37. Magaki Y, Hayasaka S, Yamada T, Hayasaka Y, Sanada M, Uonomiu T: Effects of astaxanthin on accommodation, critical flicker fusion, and pattern visual evoked potential in visual display terminal workers. J Trad Med. 19:170-173, 2002.
38. Shimada Y: Human safety study: oral dosage between 2-12 mg per day of extracted astaxanthin from H pluvialis. Fuji Chemical Industry Co., Ltd. Internal Bulletin. 2003.
39. Comhaire FH, El Garem Y, Lignell A: Presented at the Advances in infertility treatment symposium, Ft Lauderdale January 24-26, 2002 and XIII International Carotenoid Symposium Hawaii January 2002. Patent Cooperation Treaty Application W099/29313. AstaCarotene AB, Sweden.
40. Nagata N, Akiratajima, Taeko, Hamanatu, Hozukmi M: Effects of astaxanthin on metabolism during aerobic exercise of human. Toyama International Research Center for Traditional Medicine. Fuji Internal Report, 2003.
41. Wang X, Willen R, Wadstrom T: Astaxanthin-rich algal meal and vitamin C inhibit Helicobacter pylori infection in BALB/ca mice. Antimicrobial Agents and Chemotherapy. 44:2452-2457, 2000.
42. Patent Cooperation Treaty application WO98/37874. AstaCarotene AB, Sweden.
43. Maher TJ: Astaxanthin Continuing Education Module, August 2000.

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