

Memorandum

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JUN 2 2005

Date:

From: Consumer Safety Officer, Division of Dietary Supplement Programs, Office of Nutritional Products, Labeling and Dietary Supplements, HFS-810

Subject: 75-Day Premarket Notification of New Dietary Ingredients

To: Dockets Management Branch, HFA-305

Subject of the Notification: Astaxanthin present in *Haematococcus pluvialis* algal biomass

Firm: AAC Consulting Group

Date Received by FDA: March 8, 2005

90-Day Date: Mar 22, 2005

In accordance with the requirements of section 413(a) of the Federal Food, Drug, and Cosmetic Act, the attached 75-day premarket notification and related correspondence for the aforementioned substance should be placed on public display in docket number 95S-0316 as soon possible since it is past the 90-day date. Thank you for your assistance.

____Victoria Lutwak____

1995S-0316

RPT 277



Food and Drug Administration
5100 Paint Branch Parkway
College Park, Maryland 20740

James W. Barnett, Jr., Ph.D., DABT
AAC Consulting Group
7361 Calhoun Place, Suite 500
Rockville, Maryland 20855-2765

MAY 23 2005

Dear Dr. Barnett:

This is to inform you that the notification, dated March 4, 2005, you submitted on behalf of your client, FCI Health Science, Inc., a United States subsidiary of Fuji Chemical Industry Co., LTD, pursuant to 21 U.S.C. 350b(a)(2)(section 413(a)(2) of the Federal Food, Drug, and Cosmetic Act (the Act)) was filed by the Food and Drug Administration (FDA) on March 8, 2005. Your notification concerns the substance called "Astaxanthin present in *Haematococcus pluvialis* (Flowtow em. Wille) algal biomass" that you intend to market as a new dietary ingredient.

According to the notification, FCI Health Science, Inc., intends to market the new dietary ingredient "Astaxanthin present in *Haematococcus pluvialis* algal biomass in the form of tablets, capsules or incorporated into commonly used food-grade oil such as vegetable oil for use in soft-gel capsules." The notification also states that "the recommended daily intake will be 100-200 mg of *Haematococcus* algae biomass. This will result in an intake of astaxanthin of 3.8-7.6 mg/day." You indicate that under conditions of use, "the product will be labeled to notify the consumer not to exceed the recommended daily serving. The product will also be labeled that it is not intended or recommended for use by children or pregnant or lactating women."

Under 21 U.S.C. 350b(a), the manufacturer or distributor of a dietary supplement containing a new dietary ingredient that has not been present in the food supply as an article used for food in a form in which the food has not been chemically altered must submit to FDA, at least 75 days before the dietary ingredient is introduced or delivered for introduction into interstate commerce, information that is the basis on which the manufacturer or distributor has concluded that a dietary supplement containing such new dietary ingredient will reasonably be expected to be safe. FDA reviews this information to determine whether it provides an adequate basis for such a conclusion. Under section 350b(a)(2), there must be a history of use or other evidence of safety establishing that the new dietary ingredient, when used under the conditions recommended or suggested in the labeling of the dietary supplement, will reasonably be expected to be safe. If this requirement is not met, the dietary supplement is considered to be adulterated under 21 U.S.

In accordance with 21 CFR 190.6 (c), FDA must acknowledge its receipt of a notification for a new dietary ingredient. For 75 days after the filing date, your client must not introduce or deliver for introduction into interstate commerce any dietary supplement that contains the new dietary ingredient that is the subject of this notification.

Please note that acceptance of this notification for filing is a procedural matter, and thus, does not constitute a finding by FDA that the new dietary ingredient or supplement that contains the new dietary ingredient is safe or is not adulterated under 21 U.S.C. 342. FDA is not precluded from taking action in the future against any dietary supplement containing your new dietary ingredient if it is found to be unsafe, adulterated, or misbranded.

Your notification will be kept confidential for 90 days after the filing date of March 8, 2005. After the 90-day date, the notification will be placed on public display at FDA's Division of Docket Management in docket number 95S-0316. Prior to that date, you may wish to identify in writing specifically what information you believe is proprietary, trade secret or otherwise confidential for FDA's consideration.

If you have any questions concerning this matter, please contact Linda Pellicore, Ph.D. at (301) 436-2375.

Sincerely yours,



Susan J. Walker, M.D.
Director
Division of Dietary Supplement Programs
Office of Nutritional Products, Labeling
and Dietary Supplements
Center for Food Safety
and Applied Nutrition



March 23, 2005

Vickey Lutwak
Office of Nutritional Products, Labeling,
and Dietary Supplements HFS-810
Center for Food Safety and Applied Nutrition
US Food & Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740

Dear Ms. Lutwak,

This letter is in response to your request regarding the 75-day Premarket Notification for Astaxanthin present in *Haematococcus pluvialis*. You specifically requested that we provide the exact name of the new dietary ingredient. The name of the new dietary ingredient is astaxanthin present in *Haematococcus pluvialis* algae biomass

This information has been emailed to you and will be sent by fax and the original and 2 copies will be mailed to your attention as well.

Should you have any other questions, please feel free to contact me.

Sincerely,

A handwritten signature in black ink that reads 'James W. Barnett, Jr.' The signature is written in a cursive, flowing style.

James W. Barnett, Jr. Ph.D., DABT
AAC Consulting Group

772-589-4331

March 22, 2005

Vickey Lutwak
Office of Nutritional Products, Labeling, and Dietary Supplements HFS-810
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740



RE: FCI Health Science, Inc., a US subsidiary of Fuji Chemical Industry Co.
New Dietary Notification for Astaxanthin Present in *Haematococcus*
pluvialis Algae biomass

7361 Calhoun Place,
Suite 500
Rockville, Maryland 20855-2765
301.838.3120
fax: 301.838.3182

Dear Vickey:

This letter is in response to your request to Dr. James Barnett (and discussed with Dr. Stanley Tarka in Dr. Barnett's absence) for specific information regarding the 75-day Premarket Notification for Astaxanthin present in *Haematococcus pluvialis*. You specifically requested that we provide the author for *Haematococcus pluvialis* as it relates to 21§CFR190.6(b)(2) requiring the Latin binomial name (including author) of any herb or other botanical.

The information you requested as provided in National Center for Biotechnology Information located at : <http://ncbi.nlm.nih.gov> NCBI Entrez Taxonomy Database for *Haematococcus pluvialis* is as follows: ***Haematococcus pluvialis* J. Von Flotow, 1844**

Taxonomic Serial No.: 5608

Taxonomy and Nomenclature

Kingdom: Plantae
Taxonomic Rank: Species
Synonym(s):
Common Name(s):

Taxonomic

Status:
Current Standing: accepted

Data Quality

Indicators:
Record Credibility unverified
Rating:

Kingdom Plantae -- Planta, plantes, plants, Vegetal
Division Chlorophyta -- algues vertes, green algae
Class Chlorophyceae
Order Volvocales
Family Haematococcaceae
Genus Haematococcus J. Von Flotow, 1844
Species Haematococcus pluvialis J. Von Flotow, 1844

This information has been emailed to you and will be sent by via facsimile as well as an original and two copies to be mailed to your attention as well.

Should you have any other questions, please feel free to contact Dr. Barnett or myself. Thank you.

Kind regards.



Edward Steele
President

cc: Dr. James Barnett



3/09/2005

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

Re: New Dietary Ingredient Notification for Astaxanthin Present in
Haematococcus pluvialis Algal Biomass

Dear Sir/Madame:

In accordance with 21 CFR §190.6, FCI Health Science, Inc., a US subsidiary of Fuji Chemical Industry Co., LTD, is hereby notifying the FDA of its intent to market the dietary ingredient named astaxanthin as present in *Haematococcus pluvialis* algae biomass.

Should you have any questions regarding this nomenclature, feel free to call me at 512-266-2620 or e-mail to jbarnett@aacgroup.com. We will respond promptly to any questions you might have.

Best regards,

A handwritten signature in black ink that reads 'James W. Barnett Jr.' in a cursive script.

James W. Barnett, Jr., Ph.D., DABT

March 4, 2005

MAR - 8
AB/FDA

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



Re: New Dietary Ingredient Notification for Astaxanthin Present in
Haematococcus pluvialis Algal Biomass

7361 Calhoun Place,
Suite 500
Rockville, Maryland 20855-2765
301.838.3120
fax: 301.838.3182

Dear Sir/Madame:

In accordance with 21 CFR §190.6, FCI Health Science, Inc., a US subsidiary of Fuji Chemical Industry Co., LTD, is hereby notifying the FDA of its intent to market the dietary ingredient astaxanthin found at typical levels of 3.8% in *Haematococcus pluvialis* algae biomass at a daily dose up to 7.6 mg astaxanthin/day in the biomass product form.

In January 2002, Micro Gaia, Inc., now owned by Fuji, notified FDA of its intent to market the dietary ingredient astaxanthin extracted from *Haematococcus pluvialis*. In May 2002, FDA responded to this submission and concluded that astaxanthin extracted from *Haematococcus pluvialis* algae at a dose of 1-2 mg/day is reasonably expected to be safe. In a subsequent notification in March 2004, Fuji Chemical Industry Co., LTD notified FDA of its intent to market astaxanthin extracted from *Haematococcus pluvialis* algae at a dose of 2-12 mg/day. These documents, as well as new dietary ingredient notifications on astaxanthin from other companies, are referenced to docket number 95S-0316.

In this submission, we were aware of FDA's recent call for comments on the premarket notification program for new dietary ingredients (69 FR 202, PP.61680-61685; October 20, 2004). We are including additional information in this submission on the identification, analysis and manufacture of the subject dietary ingredient as well as addressing questions posed for establishing a reasonable expectation of safety.

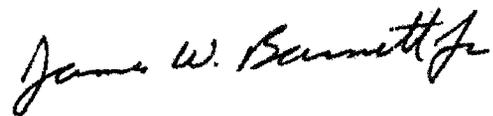
Based on the information in the following NDI submission, we believe that FDA should accept this filing on behalf of Fuji Chemical Industry Co., LTD. as providing sufficient evidence that astaxanthin in *Haematococcus pluvialis* algal biomass, when used under the conditions recommended, can reasonably be expected to be safe for human consumption.

91024

Office of Nutritional Products, Labeling and Dietary Supplements
Food and Drug Administration (CFSAN)
March 4, 2005
Page 2

Should you have any questions regarding this notification, feel free to call me at 512-266-2620 or e-mail to jbarnett@aacgroup.com. We will respond promptly to any questions you might have.

Best regards,

A handwritten signature in black ink that reads "James W. Barnett, Jr." in a cursive style.

James W. Barnett, Jr., Ph.D., DABT

Enclosures

NEW DIETARY INGREDIENT NOTIFICATION INFORMATION

I. Manufacturer

Parent Company (Japan)

Fuji Chemical Industry Co., LTD

9-11-1, Kameido, Koto-ku

Tokyo 136-8515, Japan

US Subsidiary

Charles DePrince

FCI Health Science, Inc.

18000 Horizon Way, Suite 800

Mount Laurel, NJ 08054

Phone-856-234-3636

AstaReal AB (Gustavberg, Sweden) cultivates and produces algal biomass of *Haematacoccous pluvialis* by a closed production process. The resulting product is a spray-dried powder or biomass (also referred to as algal meal). This resulting product is rich in astaxanthin (approx. 3.8%). The product is marketed under the trade name AstaREAL.

II. Chemical Identification, Manufacture and Product Analysis

Chemical Name: Astaxanthin, 3,3'-Dihydroxy-beta,beta-carotene-4,4'-dione, (3S,3'S)-

Chemical Abstracts Service Number: 472-61-7

Molecular Formula: C₄₀ H₅₂ O₄

Molecular Weight: 596.85

Source:

Haematacoccous pluvialis, phylum Chlorophyta, class Chlorophyceae, order Volvocales, family Haematococcaceae, is a ubiquitous green algae found in fresh and brackish waters. Upon

environmental stress such as drying in rock pools, the algae accumulate lipids and astaxanthin to protect against oxidation, thus becoming red in color. This is accomplished in production by limiting nitrogen and increasing light intensity in the culture. No pathogenicity or toxicity has been reported in the literature on *Haematococcus pluvialis* in Medline, Toxnet and Google searches by key words pathogenicity or toxigenicity and Haematococcus.

The production strain used in the Swedish facility is the proprietary strain ACO32 of *Haematococcus pluvialis*. During spray drying of the product, the cells of *Haematococcus pluvialis* are fractured so that the organism is no longer viable. *Haematococcus pluvialis* algal meal has been approved as a safe and acceptable biological source for the exempt-from-certification color additive astaxanthin in 21 CFR §73.185 for salmonid feeding.

Chemical Composition:

Chemical composition, nutrient levels and other analytical data for the *Haematacoccous pluvialis* algae biomass produced in Sweden are given in Attachment 1. Astaxanthin is typically present at 3.8% total astaxanthin, typically comprised of 80% monoester, 18% diester and 2% free astaxanthin in the biomass. The astaxanthin is esterified with various fatty acids. Minerals, fatty acids as triglycerides, amino acids and heavy metals content have also been analyzed and are given in Attachment 1. Also presented are aflatoxin, saxitoxin, microcystin and pesticide analyses. Data are given as available for recent analysis of product from three AstaReal V1010 batches in 2004. Most of the constituents of the algal biomass product are typical for plant or vegetable matter.

Manufacturing Process:

The *Haematacoccous pluvialis* algae are grown, harvested and dried under vigorous standards of hygiene. The algae are grown in stainless steel photobioreactors under carefully controlled hygienic conditions. The details of the production process, including process diagram and quality control procedures are presented in Attachment 2. Proprietary information on the cultivation media shows that it is generically composed of mineral salts commonly used in growth media, necessary trace nutrients, and a GRAS, food grade acid for pH control.

Manufacturing Facility:

The *Haematococcus* algae is grown at the Fuji location at the AstaReal AB facility in Gustavberg, Sweden. The AstaReal AB facility located in Sweden uses stainless steel bioreactor tanks designed to ensure sterile conditions. The facility has been inspected by the Swedish Agricultural Agency and the Environment and Health Authority and is in compliance with all rules and regulations.

III. Dietary Supplement Form, Conditions of Use and Specifications

Product Form

The final product is a fine, dark red powder with a slight algal smell. The powdered form will be supplied as a dietary supplement to be taken in the form of tablets, capsules or incorporated into commonly used food-grade oil such as vegetable oil for use in soft-gel capsules. Capsule materials, gels or any binders necessary for the final product form will be of pharmaceutical grade quality.

Product Composition

The product consists of spray-dried *Haematococcus* algae biomass containing 3.8% total astaxanthin. This equates to 3.8 mg astaxanthin/100 mg powder. The astaxanthin produced in *Haematococcus* algae is primarily the esterified 3S, 3S' astaxanthin enantiomer predominantly found in wild salmon. The astaxanthin has not been extracted from the algal biomass or in any way altered from its natural chemistry in *Haematococcus pluvialis* algae.

Dietary Supplement Conditions of Use/Labeling

The recommended daily intake will be 100-200 mg of *Haematococcus* algae biomass. This will result in an intake of astaxanthin of 3.8-7.6 mg/day. The product will be labeled to notify the consumer not to exceed the recommended daily serving. The product will also be labeled that it is not intended or recommended for use by children or pregnant or lactating women. Astaxanthin is considered to confer its beneficial health maintenance from its antioxidant properties.

Specifications

The product specifications are as follows:

Identity and carotenoid composition	>Approved
Astaxanthin %	>3.8%
Stability 5 days light	Approved
Drying loss %	<5.0%
Crushing rate	>98%
Microbiology, 10-log/gram	Approved methods
Total Fungi	Approved methods
Heavy metals	<10 ppm

The methods by which product specifications are analyzed are presented in Attachment 2.

IV. Safety Assessment of Proposed Use of Astaxanthin Dietary Supplementation

As noted in the cover letter, the proposed use of astaxanthin as a dietary supplement has been the subject of multiple NDI's in the past that are in the public record in docket number 95S-0316.

The safety data for the following submissions are thereby incorporated by reference as follows:

Fuji Chemical Industry, Ltd.	March 5, 2004
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

History of Use

Astaxanthin is a naturally present carotenoid pigment found in many animals and plants. It is responsible for the pink-to-red coloration of the flesh of various salmon species and rainbow trout, where it is present at concentrations ranging from approximately 3-40 mg/kg. It is also found at relatively high concentrations in crustacean species such as shrimp, krill, crab, lobster and crawfish. Thus, depending on the importance of seafood in the diet of various populations,

humans have ingested astaxanthin to varying degrees throughout their existence. Although the general US population has a rather low intake of about 2-3 pounds/year of salmon and an estimated intake of 29 µg/p/day astaxanthin from wild and farmed salmon, certain subpopulations such as Alaskan natives, Greenland Eskimos and Japanese fisherman have had much higher rates of salmon and seafood consumption. While no epidemiological studies have been conducted which have examined the multiple health parameters and possible effects of high dietary intake of astaxanthin *per se* in these populations, several observational studies have shown that these populations are much less susceptible to atherosclerosis, cardiovascular disease and associated sudden death than populations with lesser fish intakes (Table 1). In general, there were no negative changes in blood lipid profile, adverse clinical observations or deleterious effects on the general well being of these populations reported in these studies. Although the health benefits have been attributed to omega-3 fatty acid intake, these subpopulations were also consuming relatively high amounts of astaxanthin in the diet from wild salmon species without notable adverse effect.

Table 1. Observational studies of subpopulations with high dietary fish intake

Study reference	Study type	Outcome
Bang and Dyerberg, 1980	Observational	Ischemic heart disease accounted for only 3.5% of all deaths in Greenland Eskimos
Hirai <i>et al.</i> , 1980	Observational	Significant reduction in ischemic heart and cerebrovascular mortality in Japanese fishing villagers vs. farmers associated with higher fish intake; also reduced platelet aggregation
Kroman <i>et al.</i> , 1980	Observational	Low incidence of myocardial infarction (3 actual vs. 40 expected) in a Greenland whaling/fishing community
Kagawa <i>et al.</i> , 1982	Observational	Approximately 50% decrease in ischemic heart and cerebrovascular disease in Okinawa vs. mainland Japan
Middaugh, 1990	Observational	Annual, age adjusted death rates in Alaska Natives markedly lower than rates in non-natives (162 vs. 242; RR 0.67)
Newman <i>et al.</i> , 1993	Observational Autopsy examination of atherosclerosis	All subjects died of accidental, non-cardiovascular, causes. Native Alaskans had significantly less atherosclerosis as measured by mean percent of vessel surface with atherosclerotic lesions, fatty streaks and raised lesions than non-Natives.

Several of the prior NDI submissions on astaxanthin have utilized a hypothetical 200 gram meal of salmon to estimate likely potential upper intakes of astaxanthin. If the salmon consumed was

wild sockeye with flesh levels of 40 mg/kg, then it has been estimated that prior historical astaxanthin consumption was up to 8 mg/day. However, given that the nominal solid food consumption is 1500 g/day, and seasonal consumption of salmon may constitute a large portion of the diet in fishing and hunting subsistence communities, it is likely that salmon consumption commonly constituted more than half the diet or 700-800 g/day. This is supported by 95th percentile estimates of daily fish consumption in Northwest Indian tribes of 280-800 grams/day in a federal advisory report to EPA titled "Fish Consumption and Environmental Justice" (See Table 2 attached from NEJAC, 2002 in Consumption References given in Attachment 3). Thus, there were extended periods when these populations likely consumed 10-20 mg/day astaxanthin without any adverse effects being noted in these past studies.

Safety and Toxicology Studies

Astaxanthin, either as a purified extract from natural sources, synthetic compound or as present in algal biomass, has been evaluated in multiple animal studies to determine its potential for potential adverse effects. Its potential for genotoxicity has also been tested in several assays. The study type, study design, test material and findings are summarized in the following tables. Table 3 presents the results from studies that have been conducted on astaxanthin in algal biomass, the subject of this NDI submission. Table 4 presents the findings of studies that have been conducted on astaxanthin extracts or synthetic astaxanthin. Further details of these studies may be found in the study reports or summaries presented in Attachment 4 on astaxanthin in algal biomass and Attachment 5 on astaxanthin extracts or synthetic astaxanthin.

The lack of any toxicological findings from any study on astaxanthin are supportive of a reasonable expectation of safety from its recommended use. Astaxanthin is practically non-toxic in acute animal studies at dose up to 18 grams. When tested for genotoxicity, there was no evidence for mutagenicity in Ames/*Salmonella* assays nor did it induce micronuclei when tested *in vivo* up to 2000 mg/kg in mice. There was no maternal, embryotoxic or teratogenic effects in a teratology study in rabbits given up to 400 mg/kg over most of the gestational period. No adverse effects were noted in a one-generation reproduction study at doses up to 400 mg/kg in rats. In multiple subchronic feeding studies in rats, astaxanthin did not produce any adverse toxicological effects; there was evidence for an increase in beneficial HDL cholesterol observed in rats given synthetic astaxanthin. These studies clearly constitute a sufficient database to evaluate the potential toxicity of astaxanthin generally and as present in algal biomass.

Human Studies

Astaxanthin has been evaluated for various health effects or functional parameters in several human trials. These studies are presented in summary form in Table 5. The supporting papers or other reports are given in Attachment 6. None of the human trials have reported any notable adverse effects from intake of astaxanthin at doses as high as 40 mg/day. Several studies examined potential effects on serum clinical chemistry and hematology; the few changes noted in these parameters were generally within normal limits and not considered clinically significant. Beneficial effects on eye function and exercise tolerance have been found in repeated measures. Reductions in LDL cholesterol and reduced LDL oxidation were also reported. Therefore, the findings in human trials are considered supportive of the safety of astaxanthin supplementation alone in the diet up to levels of approximately 20 mg/day.

Safety Assessment Summary

The safety of astaxanthin in *Hematococcus pluvialis* powder has been well established. in a fairly comprehensive set of animal studies and genotoxicity assays as summarized in Table 3. No evidence for genotoxicity was found, and in subchronic feeding studies in rats, doses up to 70 mg/kg have not resulted in toxicological effects. The lack of genotoxicity or adverse effects of astaxanthin are supported by studies on *Hematococcus pluvialis* extracts or synthetic astaxanthin up to 13 weeks at doses of 162-300 mg/kg/day. In addition, the teratology and reproduction studies with synthetic astaxanthin were not associated with any treatment related developmental, teratogenic or fertility effects. The most consistent finding is some elevation in serum cholesterol and studies examining blood lipid profiles suggest that this may be due to an increase in beneficial HDL cholesterol.

The proposed dietary supplement form is composed of dried green algal meal of *Hematococcus pluvialis*. The presence of heavy metals, microbial contamination or toxins and pesticides have been analyzed and are either not found or are at low levels that are considered acceptable. Human studies and animal safety studies have shown no adverse reactions that would indicate any safety concerns with ingestion of the algal meal.

Limited trials in humans have not produced any adverse with dosing durations of 4-8 weeks at intakes ranging from 4-40 mg/kg astaxanthin/day (Table 5). These data indicate that intake of astaxanthin at 3.5-7 mg/day poses no safety concerns.

An independent expert, Harry G. Preuss M.D. of Georgetown University Medical Center, has reviewed the available literature through 2001 on the safety of astaxanthin. In his report, Dr. Preuss evaluated dietary, animal toxicity and human studies. His report concludes that astaxanthin, when used in proper doses, is safe and deserves no more safety concerns than the use of other carotenoids such as beta-carotene (Attachment 7).

For purposes of comparison, if one uses 70 mg/kg/day as a reasonable low estimate of the subchronic NOAEL and compares that to the 7 mg/day recommended upper dose in this NDI submission in a nominal 60 kg adult, the intake value of 0.1 mg/kg/day in humans has a margin-of-safety of 700 fold to the NOAEL. Typically, margins of safety of 100 are considered acceptable when regulatory bodies such as JECFA and FDA consider the safety of food additives. We think that the toxicology database is reasonably complete for this comparison to be meaningful. However, given that the total intake of carotenoids is dependent on diet and other potential supplementation, and there appears to be a threshold where skin discoloration may occur at 30-40 mg/day, an upper limit of astaxanthin supplementation of 7 mg/day is considered reasonably health protective and safe.

V. References

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- Covance Report Number 1840/002 Stewart JS: HPP: 13 week oral (dietary administration) toxicity study in the rat. (Final. pp 1-248) February 2001.
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- Kagawa, Y, Nishizawa M, Suzuki M, Miyatake T, Hamamoto T, Goto K, Motonaga E, Izumikawa H, Hirata H, Ebihara A. Eicosapolyenoic Acids of Serum Lipids of Japanese Islanders with Low Incidence of Cardiovascular Diseases. *J Nutr Sci Vitaminol*. 1982; 28: 441-453.
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Table 3. Toxicology studies conducted on *H. pluvialis* biomass

Study Type	Test Material	Dose of Biomass Duration	Species/ Assay Cells	Results	Reference
Acute oral LD ₅₀	Algal Biomass	5000 mg/kg in one dose	Rats 10/sex	No mortality or gross pathology	Technical Report #060 Cyanotech
Acute oral LD ₅₀	Algal Biomass	18000 mg/kg in one dose	Mice 10/sex	No mortality or gross pathology	Technical Report #060 Cyanotech
Acute oral LD ₅₀	Algal Biomass	12000mg/kg in split daily dose	Rats 6/sex	No mortality or gross pathology	RBM Report #950053, 1995; Fuji sponsored
Ames mutagenicity	Algal Biomass	Not available in report summary	<i>Salmonella</i> with 5 standard test strains	No increase in revertants	Technical Report #060 Cyanotech
<i>E. coli</i> mutagenicity	Algal Biomass	Not available in report summary	<i>E. coli</i> WP2 uvr A	No increase in revertants	Technical Report #060 Cyanotech
Ames mutagenicity	Algal Biomass	Up to 5000 ug/ml in plate assay	<i>Salmonella</i> with 4 standard test strains +/- S9	No increase in revertants	Scantox, #28708, 1998; Fuji sponsored
<i>E. coli</i> mutagenicity	Algal Biomass	Up to 5000 ug/ml in plate assay	<i>E. coli</i> WP2 uvr A +/- S9	No increase in revertants	Scantox Report #28708, 1998; Fuji sponsored
Micronucleus induction <i>in vivo</i>	Algal Biomass	2000 mg/kg in single dose	Mice 5/sex	No increase in micronuclei	Scantox Report #26832, 1998; Fuji sponsored
Mammalian cell mutagenicity	Algal Biomass	Up to 5000 ug/ml	Mouse lymphoma L5178Y cells	No increase in mutation frequency	Scantox Report #28709, 1998; Fuji sponsored
14 day repeat dose oral toxicity	Algal Biomass	6 g/day for 14 days	Rats 6/sex	No effects on food consumption, body weight, clinical obs., hematology, serum and urine parameters, gross or microscopic exams	RBM Report #950501, 1996 Fuji sponsored
28 day repeat dose toxicity	Algal biomass	5 or 50 mg/kg/day gavage	Rats 10/sex	No effect on clinical chemistry, hematology or gross pathology findings	MB Research Laboratories, 1999
Subchronic toxicity	Algal Biomass	1, 5 and 20% of diet as powder; for 13 weeks; estimated 1, 5 and 20 g/kg/day <i>H. pluvialis</i> powder; estimated at 3.5, 17.5 and 70 mg/kg/day astaxanthin	Rats 10/sex/dose level	No effects on food consumption, body weight, hematology, clotting, or gross pathology, clinical obs. urine/feces coloration only, urine parameters slightly altered, ↑cholesterol in mid and high dose along with ↑ alk. phos. in high dose males, ↑ renal tubular pigment in high dose females, ↓ platelets high dose, ↑ kidney weight high dose; findings not toxicologically significant; NOAEL 20 g/kg/day powder or 70 mg/kg/day astaxanthin	Covance Report # 1840/002-D6154, 2001; Fuji sponsored

Table 4. Toxicology studies conducted on astaxanthin extracts or synthetic astaxanthin

Study Type	Test Material	Dose of Astaxanthin Duration	Species/ Assay Cells	Results	Reference
Acute oral LD ₅₀	Astaxanthin	2000 mg/kg in one dose	Rats 5/sex	No mortality or gross pathology; loose stools noted	Nippon Exp. Med. Research Inst. Report # H-02227, 2002
Ames mutagenicity	Astaxanthin	Up to 5000 ug/ml in plate assay	<i>Salmonella with 4 standard test strains +/- S9</i>	No increase in revertants	Nippon Exp. Med. Research Inst. Report # H-02091, 2002
<i>E. coli</i> mutagenicity	Astaxanthin	Up to 5000 ug/ml in plate assay	<i>E. coli</i> WP2 uvr A +/- S9	No increase in revertants	Technical Report #060Cyanotech
Ames mutagenicity	Synthetic astaxanthin	Up to 5000 ug/ml in plate assay	<i>Salmonella with 5 standard test strains +/- S9</i>	No increase in revertants	Roche color additive petition 72CO211
Micronucleus induction <i>in vivo</i>	Synthetic astaxanthin	2000 mg/kg twice over 2 days	Mice	No increase in micronuclei	Roche color additive petition 72CO211
41 day repeat dose toxicity and fertility study	Astaxanthin	400 mg/kg in diet or 0.04%	Rats 8 males tox 15/sex bred	No adverse effect on growth, clinical obs, organ weights and plasma levels of liver enzymes. No effect on fertility, litter or pup size or any gross abnormalities in offspring.	Nishikawa et al., 1997
30 day repeat dose evaluation of plasma lipids	Astaxanthin	0.1% in diet	Rats 6 males	Increase in plasma HDL cholesterol	Murillo, 1992
Subchronic toxicity	Haematococcus color additive	0.025, 0.075 and 0.25% of color in diet for 13 weeks	Rats 10/sex/dose level	No effects on food consumption, body weight, hematology, organ weights or gross or microscopic pathology exams. Increase in cholesterol at high dose level only noted serum chemistry change. NOAEL-0.25%,	Ono et al., 1999
Teratology	Synthetic astaxanthin	100, 200 or 400 mg/kg/day gestation days 7-19	Rabbits	No maternal toxicity, pup size and litters unchanged, slight increase in resorptions at high dose, no malformations associated with treatment,	Roche color additive petition 72CO211
One-generation reproduction	Synthetic astaxanthin	25, 100 or 400 mg/kg/day Males 70 days prior to mating; Females 14 days prior to mating	Rats 32 /sex/group	No effects on any fertility parameter or measures of pup size, litter size, growth or fetal development	Roche color additive petition 72CO211

Table 4. Toxicology studies conducted on astaxanthin extracts or synthetic astaxanthin

Study Type	Test Material	Dose of Astaxanthin Duration	Species/ Assay Cells	Results	Reference
Subchronic toxicity	Synthetic astaxanthin	310, 620 and 1240 mg/kg/day from diet for 13 weeks	Rats	No effect on growth, Decrease in organ weight observed in mid and high dose in kidney, ovary, adrenals, uterus, and spleen. Clinical chemistry normal with exception of elevated cholesterol in all groups.	Roche color additive petition 72CO211
Subchronic toxicity	Synthetic astaxanthin	41, 76 and 162 mg/kg/day intake in diet for 13 weeks	Dogs 3/sex/dose	No adverse effects on body weight, clinical obs, hematology, clinical chemistry, gross necropsy or histopathological exam s	Roche color additive petition 72CO211

Table 5. Human studies with astaxanthin

Study Type/ Parameters	Dose of Astaxanthin/ Duration	Number of Subjects	Results	Reference
LDL oxidation	8, 3.6, 14.4 or 21.6 mg/day for 2 weeks	5, 5, 3 and 5 respectively	No adverse reactions; reduction in LDL oxidation	Iwamoto et al., 2000
LDL oxidation	3.6, 7.2 or 14.4 mg/day for 2 weeks	5, 5 and 3 respectively	No adverse reactions; reduction in LDL oxidation	Miki et al., 1998 Patent 10155459
Safety assessment	3.85 or 19.5 mg/day for 29 days	33	No adverse effect on weight, skin color, appearance, B.P, vision, depth perception, ENT condition, oral cavity appearance, chest and lungs, reflexes, serum chemistry, hematology and urinalysis	Mera Pharmaceuticals Report, 1999
Safety assessment	2, 4 or 12 mg/day for 4 weeks	4, 7 or 6 respectively	No adverse reactions; no treatment related effects of serum biochemistry or hematology	Shimada, 2003
Safety assessment	6 mg/day for 8 weeks	19	No effects on clinical chemistry, blood pressure and blood counts; increased serum calcium and protein and eosinophil count; not considered clinically significant	Spiller and Dewell, 2003
Eye function	6 mg/day for 4 weeks	9	No adverse effects noted; improved deep vision and critical flicker fusion	Keisuke et al., 2002s
Eye function	5 mg/day for 4 weeks	13	No adverse reactions; improved resistance to eye fatigue in video terminal workers	Magaki et al, 2002
Exercise tolerance	4 mg/day for 6 months	20	No adverse reactions; increase in strength endurance	Lignell et al., 1999 Patent WO98137874 Malmsten, undated
Exercise tolerance	5 mg/day for 2 weeks	9	No adverse reaction noted; LDL cholesterol reduction; improved respiratory efficacy	Nagata et al., 2003
Plasma lipids and peroxidation	8 mg/day for 12 weeks	20 males placebo and treated	Marked increase in plasma astaxanthin levels; no change in plasma vitamin A or C equivalents, lycopene or other carotenoids, no change in interleukin markers; no change in serum lipids or fatty acid profile; significant decrease in fatty acid peroxidation	Salonen, 2001
Dyspepsia treatment	16 or 40 mg/day astaxanthin in algal meal for 4 weeks	44-placebo 43 low dose 44-high dose	Treatment with 40 mg/day reduced reflux; no adverse effects reported	Kupcinskas et al., 2003

ATTACHMENT 1

Tables of Analyses

Fatty Acid Content

Nutrition

Amino Acid Analysis

Minerals and Heavy Metals

Toxins and Pesticides

Carotenoid Content

Chemical composition of AstaCarox V1010
(Typical Analysis Results)

<i>Nutrients</i>	<i>Unit</i>	<i>Value</i>	<i>Amino acids</i>	<i>Unit</i>	<i>Value</i>
Moisture	%	4.5	Aspartic acid	mg/g	7.2
Fat, acid hydrolysis	%	42	Threonine	mg/g	4.4
Protein, Dumas	%	9.5	Serine	mg/g	4.8
Total Carbohydrate	%	40	Glutamic acid	mg/g	9.7
Dietary fiber	%	27	Proline	mg/g	4.3
Ash	%	3.9	Glycine	mg/g	5.2
Calories	Cal/100 g	502	Alanine	mg/g	8.0
Calories from fat	Cal/100 g	254	Cystine	mg/g	1.1
Cholesterol	mg/100 g	<1	Valine	mg/g	5.2
			Methionine	mg/g	1.9
<i>Fatty acids</i>	<i>Unit</i>	<i>Value</i>	Isoleucine	mg/g	3.2
C 14:0	%	0.11	Leucine	mg/g	7.7
C 16:0	%	5.0	Tyrosine	mg/g	2.6
C 17:1	%	1.0	Phenylalanine	mg/g	3.8
C 18:0	%	1.0	Histidine	mg/g	1.8
C 18:1	%	8.9	Lysine	mg/g	4.6
C 18:2, n-6	%	8.1	Arginine	mg/g	4.9
C 18:3, n-6	%	0.37	Tryptophan	mg/g	1.2
C 18:3, n-3	%	3.3			
C 20:0	%	0.16	<i>Minerals</i>	<i>Unit</i>	<i>Value</i>
C 20:1	%	0.06	Calcium	ppm	13 500
C 20:2, n-6	%	<0.04	Copper	ppm	4.4
C 20:4, n-6	%	0.24	Iron	ppm	100
C 20:5, n-3	%	0.06	Magnesium	ppm	350
C 22:0	%	0.05	Manganese	ppm	70
C 22:6	%	<0.04	Phosphorus	ppm	2 400
			Potassium	ppm	2 300
<i>Fatty acid composition</i>	<i>Unit</i>	<i>Value</i>	Sodium	ppm	260
Omega 3	%	3.3	Zinc	ppm	13
Omega 6	%	8.8	Aluminum	ppm	27
Saturated fat	%	6.0	Chromium	ppm	5
Monounsaturated fat	%	9.5	Iodine	ppm	0.4
Polyunsaturated fat	%	11.6	Selenium	ppm	<0.03
<i>Carotenoid composition</i>	<i>Unit</i>	<i>Value</i>	<i>Heavy metals</i>	<i>Unit</i>	<i>Value</i>
Total carotenoids	%	4.0	Arsenic	ppm	<0.05
Astaxanthin -	%	3.8	Cadmium	ppm	0.01
as monoester	%	3.1	Lead	ppm	0.03
as diester	%	0.7	Mercury	ppm	<0.02
free	%	0.05			
Lutein	%	0.02	<i>Aflatoxin</i>	<i>Unit</i>	<i>Value</i>
Canthaxanthin	%	0.02	B1	ppb	<0.5
Adonirubin	%	0.02	B2	ppb	<0.5
β -carotene	%	0.02	G1	ppb	<0.5
			G2	ppb	<0.5
			<i>General pesticides</i>	<i>Unit</i>	<i>Value</i>
			Organophosphates	ppm	ND, <0.05
			Organonitrogens	ppm	ND, <0.50
			Organochlorinated	ppm	ND, <0.20

The values represent the average of analysis results on three batches: ASR 239 (200 kg), ASR 040920R (28 kg), and ASR 241 (200 kg) produced in May, September and December 2004 and thus should well cover any variation in the production process and can be considered as typical.

ATTACHMENT 2

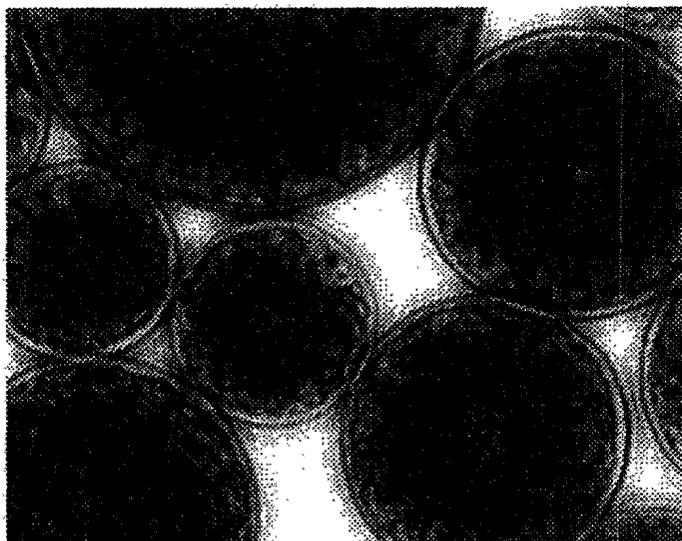
**Production Process, Process Diagram, Quality Control Procedures
Specifications**

2005-02-28



AstaCarox

Production, processing and quality control procedures



PAGES 23 THROUGH 28

REDACTED IN ITS
ENTIRETY
CONTAINS
TRADE SECRET
CONFIDENTIAL
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INFORMATION

ATTACHMENT 3

**Referenced Papers for Consumption Analysis
As listed/ordered in Table 1**

**Table 1 From NEJAC , 2002 online at
http://www.epa.gov/compliance/resources/publications/ej/fish_consump_report_1102.pdf**