

**Memorandum**

1859 5 JUN 30 P2:17

Date:

JUN 15 2005

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: *Haematococcus pluvialis* algal

Firm: U.S. Nutra, LLC

Date Received by FDA: \_\_\_\_\_ March 18, 2005

90-Day Date: June 16, 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

RPT278



Mr. Anthony Young  
Kleinfeld, Kaplin and Becker, LLP  
for U.S. Nutra, LLC  
1140 Nineteenth Street, N.W.  
Washington, DC 20036-6606

JUN 3 2005

Dear Mr. Young:

This is to inform you that the notification, dated March 18, 2005, you submitted on behalf of your client, U.S. Nutra, LLC, 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 18, 2005. Your notification concerns the substance that you call "Zanthin extract Astaxanthin complex from *Haematococcus pluvialis* (flotow em. Wille) algal" that you intend to market as a new dietary ingredient.

According to the notification, U.S. Nutra, intends to market the new dietary ingredient, "Zanthin extract Astaxanthin complex from *Haematococcus pluvialis* (flotow em. Wille) algal", in the form of tablets and capsules. The notification also states that "the recommended daily intake will be 50 mg per day".

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

21U.S.C. 342(f)(1)(B) because there is inadequate information to provide reasonable assurance that the new dietary ingredient does not present a significant or unreasonable risk of illness or injury.

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 10, 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 further questions concerning this matter, please contact Linda S. Pellicore, Ph.D., at (301) 436-2375.

Sincerely yours,



*for* 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

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March 17, 2005

Via Overnight Mail

Office of Nutritional Products,  
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Center for Food Safety and Applied Nutrition  
Food and Drug Administration  
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A.B./FDA

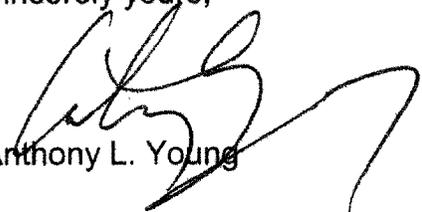
RE: New Dietary Ingredient Notification  
U.S. Nutra, LLC

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. Nutra, LLC, Eustis, Florida. Please call, write or e-mail the undersigned ([ayoung@kkblaw.com](mailto:ayoung@kkblaw.com)), who is designated by U.S. Nutra, should you have any questions regarding this submission.

Please note that Appendices 4, 5, 8, 18 and 19 constitute trade secret and confidential commercial information which U.S. Nutra does not disclose outside the Company, except to those with whom it has a confidential relationship. Each of these appendices is marked Confidential and is provided in a separate envelope similarly marked

Sincerely yours,

  
Anthony L. Young

Enclosures

OTS  
9/125

## 75 Day Pre-Market Notification

### 1. Manufacturer:

U.S. Nutraceuticals, LLC dba U.S. Nutra  
2751 Nutra Lane  
Eustis, FL 32726

### 2. New Dietary Ingredient:

Zanthin<sup>®</sup> Extract Astaxanthin Complex – 10 percent Standardized, derived from *Haematococcus pluvialis* Flotow emend. Wille

### 3. Conditions of Use

As an ingredient for use in dietary supplements at levels not to exceed 50 mg Zanthin<sup>®</sup> Extract Astaxanthin Complex per day [Equivalent to 5 mg astaxanthin].

### 4. Basis for Conclusion that Zanthin<sup>®</sup> Extract Astaxanthin Complex – 10 percent Standardized is Reasonably Expected to be Safe Under Conditions of Use;

This Submission was Prepared by: Herbal Sciences International Ltd, The Seed Bed Centre, Langston Road, Loughton Essex, IG10 3TQ, UK. Contact person: Dr John Wilkinson PhD Email: [JW1@fsmail.net](mailto:JW1@fsmail.net)

## Introduction

Zanthin<sup>®</sup> Extract Astaxanthin Complex is produced by extraction with supercritical carbon dioxide, of the dried algae *Haematococcus pluvialis*. This extract is produced by U.S. Nutra at their manufacturing facility in Eustis, Florida. This extract contains astaxanthin-based carotenoids which are responsible for the pink coloration of the flesh of salmonids, and crustaceans such as and lobsters.

*Haematococcus pluvialis*, when cultured and then subjected to environmental stress, encysts and accumulates astaxanthin, probably as protection against damage from ultraviolet light, and it is then harvested at this stage for optimal Astaxanthin content. In dried 'cracked' form, *Haematococcus pluvialis* has been marketed as a new dietary ingredient in dietary supplements without FDA objection, by several companies, such as Aquasearch, Inc. (marketed as 'Astafactor', see Aquasearch 2000i and 2000ii) and Cyanotech (marketed as BioAstin, e.g., Purecaps 2004). Similar dried algal preparations have also been marketed as dietary supplements in the European Union since prior to 1997.

In March 2004, U.S. Nutra was the first company to obtain novel foods approval for its CO<sub>2</sub> extract of *Haematococcus pluvialis* for sale in the EU as a dietary

supplement (FSA 2004).

It is U.S. Nutra's submission that **Zanthin<sup>®</sup> Extract Astaxanthin Complex** is reasonably expected to be safe and that this ingredient is substantially equivalent to other *Haematococcus pluvialis* products (both of the dried algae, and of its extracts) already marketed within the United States as new dietary ingredients. The basis for this conclusion is presented in this report.

*It should be noted that a number of mergers and takeovers have occurred amongst the suppliers of Haematococcus - derived products. Fuji have acquired Bio Process AB (formerly Astacarotene AB) in Sweden, their astaxanthin products are now traded under the name AstaReal. Fuji have also acquired Micro Gaia, in Hawaii, their astaxanthin is traded as BioReal. Mera and Aquaseach have merged and trade astaxanthin as AstaFactor<sup>®</sup>. Cyanotech appear to be intact and trade their astaxanthin (again for the human market) as BioAstin<sup>®</sup>. For the purposes of this report, the corporate name considered most appropriate for clarity will be used, even if this name is no longer current.*

## **Section 1- Astaxanthin: Its Chemistry, Occurrence and Origin.**

**Section 2- *Haematococcus pluvialis*, Description, Cultivation and Properties**

**Section 3- Preparation of Dried Algal Meal**

**Section 4- Composition of Zanthin<sup>®</sup> Extract Astaxanthin Complex**

**Section 5 - Demonstration of Substantial Equivalence of Zanthin<sup>®</sup> Astaxanthin Rich Oleoresin Zanthin<sup>®</sup> Extract Astaxanthin Complex and Dried Algal Meal**

**Section 6- Effects of the Production Process Used to Produce Zanthin<sup>®</sup> Extract Astaxanthin Complex.**

**Section 7- Preparation of Zanthin<sup>®</sup> Extract Astaxanthin Complex from Dried *Haematococcus pluvialis* meal**

**Section 8- Stability of *Haematococcus* preparations: Algae, Oleoresin and Capsules**

**Section 9- History of the Use of *Haematococcus Pluvialis*, the source of Zanthin<sup>®</sup> Extract Astaxanthin Complex**

**Section 10- Anticipated levels of Intake and Usage of Zanthin<sup>®</sup> Extract Astaxanthin Complex**

**Section 11- Review of Human Consumption of Astaxanthin-Containing Products and Foodstuffs**

**Section 12- Microbiological Information on Zanthin<sup>®</sup> Extract Astaxanthin Complex**

**Section 13- Safety, Bioavailability and Toxicity Studies on *Haematococcus*-derived Astaxanthin Products**

**Section 14- Conclusion**

**Section 15- References**

**Section 16-List and Guide to Appendices**

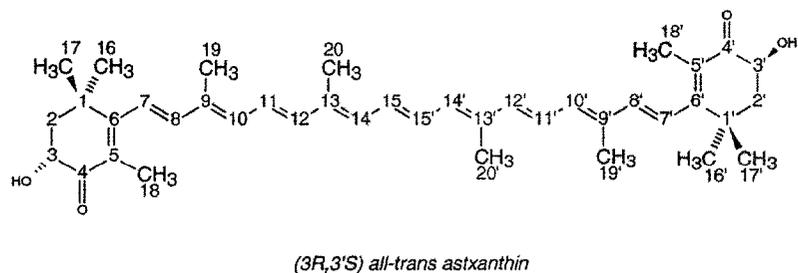
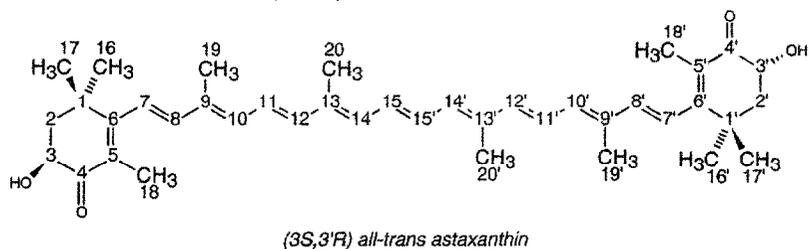
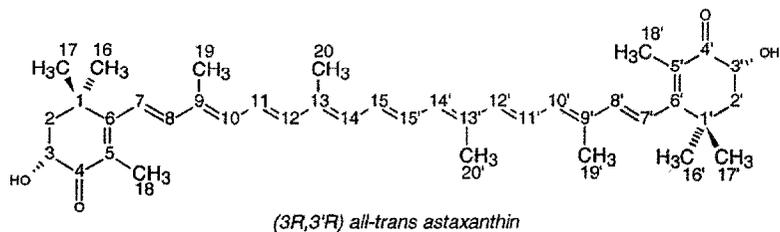
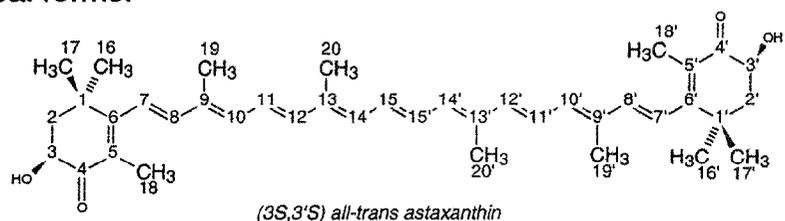
**Appendices**

**Section 1**

**Astaxanthin: Its Chemistry, Occurrence and Origin.**

Chemically, astaxanthin is *3,3'-dihydroxy-β,β-carotene-4,4'-dione*, (CAS 471-53-4), and is a naturally occurring carotenoid found in many organisms, e.g. salmon, trout, lobsters and shrimps (Maher 2000, Wilkinson 2004), and has been shown to be responsible for the pink coloration in the flesh of these creatures (Turujman *et al.* 1997). It has been demonstrated (Foss *et al.* 1984) that astaxanthin is not synthesised *de novo* in salmonids, but is obtained entirely from dietary sources, principally via ingestion of krill (a small crustacean) and micro-algae. Astaxanthin is also found and biosynthesized in the yeast *Phaffia rhodozyma*, and the micro-algae *Haematococcus pluvialis* which is the subject of this report.

Astaxanthin is a member of the carotenoid family, and exists in several stereochemical forms:



The isomeric distribution of these forms varies according to the source, as shown in Table 1

**Table 1 Distribution of Astaxanthin stereoisomers in selected organisms (Bjerkeng 1997)**

SPECIES	(3S,3'S)	(3R,3'R) and (3R,3'S)	(3R,3'R)
Yeast ( <i>Phaffia sp.</i> )	---	<2%	>98%

SPECIES	(3S,3'S)	(3R,3'R) and (3R,3'S)	(3R,3'R)
Micro algae ( <i>Haematococcus</i> )	100%	---	---
Synthetic Astaxanthin (Carophyll Pink La Roche)	25%	50%	25%
Atlantic Salmon	78-85%	2-6%	12-17% (Schiedt et al 1981) cited in Foss 1984

It can be seen that the algae *Haematococcus pluvialis* (the source of Zanthin®) biosynthesizes astaxanthin in the pure 3S, 3'S form with virtual optical purity. This is the isomer which predominates in the astaxanthin occurring in salmon species (Schiedt *et al.*, 1981 cited in Foss 1984). Astaxanthin is found in nature in both the *cis*- and *trans*- isomeric forms in varying proportion; these forms are readily interconvertible under mild conditions (Chen *et al.* 1999). Free astaxanthin occurs in limited quantity in nature, and is more commonly found in organisms bound as its mono- or di-esters with fatty acids (Bjerkeng *et al.*, 1997, Renstrom *et al.*, 1981).

## Section 2

### **Haematococcus pluvialis, Description, Cultivation and Properties**

*Haematococcus pluvialis* is a unicellular, green, freshwater algae of wide occurrence. Taxonomically it may be described as *Haematococcus pluvialis*, of the family *Haematococcaceae*, the order *Volvocales*, the class *Chlorophyceae* and the division *Chlorophyta*. For convenience and brevity, in this report, the terms *Haematococcus* and *Haematococcus pluvialis* will be used interchangeably to describe this organism.

Under conditions of environmental stress (e.g. high light intensity, nutrient deprivation), the green cells differentiate from a vegetative stage to form aplanospores in a resting stage. This, referred to as the 'red cyst' stage by the cultivators, is accompanied by an increase in cell volume and formation of a hard cellular wall that accumulates astaxanthin and its fatty acid derivatives (Boussiba *et al.*, 1992, Cohen *et al.*, 2002). It is believed that *Haematococcus pluvialis* biosynthesizes astaxanthin as a pigment, effectively a sunscreen, to protect the cells against UV damage (Kobayashi *et al.* 1992).

## Section 3

### **Preparation of Dried Algal Meal**

The standard procedure for the production of a stable, nutritionally bio-available form of *Haematococcus pluvialis* is as follows:

The algae is cultured either in open ponds or bioreactors (Wiener *et al.* 2003), then, at the 'red cyst' stage (*i.e.* the stage of maximal astaxanthin content), the algae is harvested and then dried. The dried material is then 'cracked' (*i.e.* fragmented to crack open the cells) to enable the secondary metabolites to be in a bio-available form. This material is subsequently vacuum-packed, and supplied in this form, either for direct encapsulation/ tablet production, or else to manufacturers (such as U.S. Nutra) for the preparation of standardized extracts of *Haematococcus*-derived astaxanthin. The cracked, dried preparation (formulated with preservatives and other materials) is the 'dried algal meal' marketed by various companies (e.g. Cyanotech and Mera) as a nutritional supplement.

Analysis of the carotenoid fraction of cultured *Haematococcus pluvialis* (Renstrom *et al.*, 1981) gives the following composition:-

<b>Total astaxanthins</b>	<b>84%*</b>
<b><math>\beta,\beta</math>-Carotene</b>	<b>1%</b>
<b>Adonirubin (as ester)</b>	<b>3%</b>
<b>Lutein</b>	<b>7%</b>
<b>Violaxanthin</b>	<b>2%</b>
<b>Neoxanthin</b>	<b>1%</b>

\* (comprising 1% as free astaxanthin, 7% bound as a diester, 76% bound as a monoester)

This correlates closely with earlier published work on this algae (Renstrom 1981 and references therein).

Analyses of the carotenoid fractions of commercially produced *Haematococcus pluvialis* show a closely similar pattern with respect to the range and quantity of carotenoid production, indicating that, despite differences in both culturing methodologies and specific strains of *Haematococcus pluvialis*, the phytochemical profile of the carotenoid content of the algae from different sources is very similar. (See Appendix 20 and Section 6)

Over a representative range of samples of *Haematococcus pluvialis* taken from various sources (Aquasearch 2000i, p12 and refs therein) the variations are as follows:-

**Astaxanthin (total) 81-99% as (Free astaxanthin 1-5%; astaxanthin monoesters 46-79%; astaxanthin diesters 10-39%)**  
 **$\beta$ -carotene 0-5%**  
**lutein 1-11%**  
**canthaxanthin 0-5.5%**  
**other carotenoids 1-9%**

The E/Z ratio of the astaxanthin isomers is similarly little affected by the source of the algae.

**Table 2. E/Z ratios of Astaxanthin isomers in various sources of *Haematococcus* algae**

Source of algae	All - E Astaxanthin	9-Z Astaxanthin	13-Z Astaxanthin
Aquasearch <sup>1</sup>	1.30	0.10	0.20
Aquasearch <sup>1</sup>	1.90	0.30	0.30
Aquasearch <sup>1</sup>	2.10	0.40	0.40
U.S. Nutra <sup>2</sup>	2.88	0.48	0.68

Figures expressed as % of Total Astaxanthin. References: 1. Aquasearch 2000i, page 89 ; 2. See appendix 2

## Section 4

### Composition of Zanthin<sup>®</sup> Extract Astaxanthin Complex

**Zanthin<sup>®</sup> Extract Astaxanthin Complex** is an extract (using supercritical carbon dioxide as solvent) of the dried *Haematococcus pluvialis* algae. Supercritical carbon dioxide is widely used in the food industry to extract, for example, caffeine from coffee beans and flavour components from hops. U.S. Nutra's extraction uses pure supercritical carbon dioxide, without the use of additional organic entrainers or co-solvents. Upon solvent removal, this gives an oleoresin of the lipid-soluble components of the crude algae, composed primarily of the carotenoids and the higher (more lipophilic) fatty acids. Typically, 30% of the weight of dried algae is recovered as oleoresin. This material contains (typically) 10.2% of total carotenoids, the balance being lipids and water (Appendix 1).

### Table 3 Fatty acids composition

		<b>CO<sub>2</sub> Extract<sup>1</sup></b>
		% of total FA
12:0	Lauric	0.07
13:0	Tridecanoic	0.10
14:0	Myristic	0.51
15:0	Pentadecanoic	0.03
16:0	Palmitic	12.21
16:1 n-7	<i>cis</i> -9-Palmitoleic	0.32
17:0	Heptadecanoic	0.11
17:1	<i>cis</i> -10-Heptadecenoic	1.87
18:0	Stearic	0.79
18:1 n-9 <i>clt</i>	<i>cis</i> -9-Oleic and/or <i>trans</i> -9-Elaidic	24.14
18:2 n-6 <i>clt</i>	<i>cis</i> -9,12-Linoleic and/or <i>trans</i> -9,12-Linolelaidic	30.68
20:0	Arachidic	1.77
18:3 n-6	$\kappa$ -Linolenic	14.83
20:1	<i>cis</i> -11-Eicosenoic	0.25
18:3 n-3	Linolenic	0.18
21:0	Heneicosanoic	1.65
20:2	<i>cis</i> -11,14-Eicosadienoic	0.48
22:0	Behenic	0.06
20:3 n-6	<i>cis</i> -8,11,14-Eicosatrienoic	1.34
22:1 n-9	<i>cis</i> -13-Erucic	0.06
20:3 n-3	<i>cis</i> -11,14,17-Eicosatrienoic	8.37
20:4 n-6	<i>cis</i> -5,8,11,14-Arachidonic	0.12
20:5 n-3	<i>cis</i> -5,8,11,14,17-Eicosapentaenoic	0.06

**Table 4. Carotenoid composition of CO<sub>2</sub> extract<sup>1</sup>**

	CO <sub>2</sub> Extract <sup>1</sup>	
	% w/w	% of total
E-Astaxanthin	5.92	59
9Z-Astaxanthin	1.48	15
13Z-Astaxanthin	2.58	26
Total Astaxanthins	9.98	99
B-Carotene	0.03	0.3
Lutein	0.07	0.7
Canthaxanthin	0.03	0.3
Total Other Carotenoids	0.13	1.3
Total Carotenoids	10.1	

1. Data derived from appendix 2

## **Section 5**

### **Demonstration of Substantial Equivalence of Zanthin® Extract Astaxanthin Complex and Dried Algal Meal**

Astaxanthin has been approved and is widely used as a feed ingredient for fish-farmed salmon; so that its presence in the human food chain is clearly established. In addition, many companies worldwide (e.g. Mera Pharmaceuticals (Mera 2000, 2004i 2004ii), and Cyanotech (Sold as BiAstin by Nutrex-Hawaii (2004), Purecaps (2004)) have marketed preparations of *naturally derived* astaxanthin (i.e. from *Haematococcus* and other sources) as human dietary supplements.

A number of experiments have been conducted by U.S. Nutra in order to show the phytochemical equivalence of the dried *Haematococcus* algae (sold as a dietary supplement in the US and EU), and U.S. Nutra's supercritical extract of *Haematococcus pluvialis*. In order to demonstrate the substantial phytochemical equivalence of dried *Haematococcus pluvialis*, U.S. Nutra has performed a number of analyses of the oleoresin and of the raw algal meal. The methods used were as follows:-

The sample of algal meal (from Lot AST10203) was supplied by ALGA technologies Ltd

The sample of U.S. Nutra's **Zanthin® Extract Astaxanthin Complex** is taken from Lot 031029HAOR.

There is no 'officially specified' method of analysis for carotenoids in *Haematococcus* at present, therefore the methods employed were developed 'in-house' by U.S. Nutra, combining spectrophotometric and HPLC techniques, using published analytical methods and emerging industry standards as guidelines. The actual analyses were all performed (in duplicate) by U.S. Nutra's

'in-house' scientists. The fatty acids were analysed by GC according to the method of the US Pharmacopoeia. The analytical protocols and supporting references from the scientific literature are included in Appendix 3.

### **Carotenoid Determination in Zanthin® Extract Astaxanthin Complex and in the Dried Algal Meal**

To test for total astaxanthin content, **Zanthin® Extract Astaxanthin Complex** was enzymatically hydrolysed with cholesterol esterase to convert all available astaxanthin to the 'free' form, then analysed by HPLC on a normal-phase silica column. To determine other carotenoids, the extracts were subjected to HPLC directly, without prior hydrolysis. A C30 column was used for determination of lutein and canthaxanthin, and a C18 column for  $\beta$ -carotene.

The algal meal was first extracted with acetone (approx 1:500 w/v acetone/algae) and the solution subjected to HPLC as described above.

Full details of the protocols for analysis of both the algal meal and the oleoresin are supplied in Appendix 3.

### **Results**

Comparative results for fatty-acid, and carotenoid content for both the dried algae and the supercritical CO<sub>2</sub> extract are presented in Appendix 2.

Twenty three identifiable fatty acids were determined and reported on a w/w basis. All fatty acids were found in similar proportions in both the algal meal and the supercritical CO<sub>2</sub> extract. The total carotenoid content was increased from 4.1% overall in the raw algal meal, to 10.1% overall in the extract, due to removal of CO<sub>2</sub>-insoluble biomass (Higher carbohydrates, proteins, mineral salts etc). The relative proportions of the total astaxanthin and other carotenoids remained virtually unchanged from the algal meal to the extract.

The minor change in the E-Z ratio of the astaxanthin should be noted. E- to Z-transformation has been documented for astaxanthin under solvation, and heat treatment (Chen *et al.* 1999), so this minor change is to be expected even under the extremely mild conditions of supercritical CO<sub>2</sub> extraction. Toxicology issues relating to this slight change in this geometric isomer ratio are discussed in Section 13, but are considered to be of insignificant consequence.

Finally U.S. Nutra also analysed Mera Pharmaceuticals *Haematococcus pluvialis* algae, since this product has been on sale as a new dietary ingredient in the United States for a number of years and has not been objected to by the FDA (Aquasearch 2000i and 2000ii). The results detailed in appendix 13, show that both Algatechnologies (the source that U.S. Nutra use for extraction purposes) and Mera Pharmaceuticals dried algae did not have any significant differences in terms of their astaxanthin content.

## **Conclusion with Respect to Substantial Equivalence**

The substantial equivalence of **Zanthin<sup>®</sup> Extract Astaxanthin Complex** and the dried *Haematococcus pluvialis* algal meal with respect to the phytochemical composition (i.e. the biologically active components) is clearly demonstrated by the above results; the primary difference being the higher total content of lipids and carotenoids in the oleoresin relative to the algal meal. The **Zanthin<sup>®</sup> Extract Astaxanthin Complex** will subsequently be diluted to provide a maximum content of 5mg Astaxanthin per capsule. This level is comparable with those in other manufactures substantially equivalent astaxanthin-based nutritional supplements, see Section 10.

The above results show the substantial equivalence of U.S. Nutra's **Zanthin<sup>®</sup> Extract Astaxanthin Complex** with regard to its phytochemical profile to that of both ALGAtchnologies and Mera Pharmaceuticals algae.

## **Section 6**

### **Effects of the Production Process Used to Produce Zanthin<sup>®</sup> Extract Astaxanthin Complex**

The overall production process has already been outlined; the algae is cultured, harvested at the stage of maximum astaxanthin content, dried and 'cracked'. The algal meal is then extracted with supercritical carbon dioxide, and this solvent subsequently removed to give the final product.

### **Worldwide Methods of Production of *Haematococcus pluvialis*.**

*Haematococcus pluvialis* is produced by a number of companies worldwide, by slightly different methods (Wiener *et al* 2003) *e.g.* -

- i) Cyanotech use an 'open pond' system in the USA
- ii) Fuji Chemical Industries uses an indoor system in Sweden (formerly owned by Astacarotene AB).
- iii) Mera has dome-shaped bioreactor facilities in Hawaii
- iv) ALGAtchnologies uses solar-powered bioreactors in a closed, closely controlled system at their facilities in Israel

The algal meal used by U.S. Nutra for the preparation of Zanthin<sup>®</sup> at their Florida (USA) plant is supplied by ALGAtchnologies Ltd (based in Israel). The production method employed is detailed in Appendix 4 (**Confidential**). Similarly, the exact source and strain of *Haematococcus* used by ALGAtchnologies is confidential (See Appendix 5 (**Confidential**) for details of strain, also Appendix 6 for Certificate of Analysis, and Appendix 7 for Material Safety data sheet) , but most companies

worldwide use one of the following strains of *Haematococcus* :-

***Haematococcus* Strains & Species** (From Cohen 2000)

**Strains & Species Source**

*H. pluvialis* flotow CCAG, Gottingen

*H. pluvialis* flotow NIES Tsukuba, Japan

*H. lacustris* UTEX 16 CCAT, USA

*H. pluvialis* flotow ETTL 1958/3 Ceska republika

*H. pluvialis* Flo-TAKAOOVA 1983/1 Same as above

*H. Droebicensis* CCAP 43/2G CCAP, UK

*H. pluvialis* Flo. 1844 em. Willie K-0084 SCCAP, Denmark

Despite the fact that the precise strains of *Haematococcus* used by the various companies worldwide are commercial secrets, it is generally accepted (Evans 2001) that the phytochemical profiles of the same species of plants (including algae), are often very similar, although this may vary with the technique of cultivation, climatic conditions, and other factors. In the case of *Haematococcus Pluvialis*, expert opinion (Appendix 20) supports the view that the phytochemical products of different strains of *Haematococcus* algae, will be very similar if the same growing condition and manufacturing condition are applied. Appendix 20 details the opinions of Dr. M. Olaizola (Mera Pharmaceuticals) and Dr. A Drory (ALGAtotechnologies) concerning the equivalence of their own products with that of Astacarotene in Sweden. Summarized, two separate manufacturers of *Haematococcus* products (produced from their favoured strains of *Haematococcus pluvialis*), based in Israel (ALGAtotechnologies) and Hawaii (Mera) respectively, take the view that both their algae, their production methods and their products are substantially equivalent to the algae, methods and products of a third producer (Astacarotene, now Fuji), whose products are marketed in EU as Astaxin, and whose production facility is based in Sweden.

The analytical data in Appendix 13 provides yet further support to this view, showing analyses of *Haematococcus* from ALGAtotechnologies (grown in Israel), and Mera (grown in Hawaii).

**Section 7**

**Preparation of Zanthin<sup>®</sup> Extract Astaxanthin Complex from Dried *Haematococcus pluvialis* meal.**

The algae is supplied by ALGAtotechnologies to U.S. Nutra in dried, cracked, vacuum-packed form. It should be noted that preparations of this algal meal are already marketed without FDA objection in the U.S. (e.g. by Cyanotech) and by Aquasearch (2000i, 2000ii) as human nutritional supplement. Prior to extraction, the algal meal contains around 4% (+/- 1%) of total astaxanthin (See Appendix 7). The extraction process with supercritical CO<sub>2</sub> is carried out at U.S. Nutra's Florida plant, and yields approx 30% (based on starting mass of dried algal meal) of a dark red oleoresin. This resin contains approx 10% of carotenoids, and approximately

83% of this carotenoid fraction is astaxanthin (mixed isomers) in both free- and ester form.

Full details of the methods of production employed for preparing *Haematococcus* and extraction of the astaxanthin-rich oleoresin are to be found in Appendix 8 (**Confidential**). This oleoresin is combined with previous batches of extract to produce a consistent, standardized product of 10% astaxanthin content, and this material is defined as "**astaxanthin-rich carotenoid oleoresin**" or **Zanthin® Extract Astaxanthin Complex**. This oleoresin, freed by the extraction process from proteins, carbohydrates and minerals will be used to produce capsules and tablets containing up to 5mg total astaxanthin, by dietary supplement manufacturers, diluted with suitable excipients *e.g.* food-grade oils.

The exact composition of **Zanthin® Extract Astaxanthin Complex** is found in the following appendices:-

Appendix 1 - Certificate of Analysis of **Zanthin® Extract Astaxanthin Complex**;  
Appendix 2 - Fatty acid and carotenoid analysis of **Zanthin® Extract Astaxanthin Complex**;  
Appendix 3 - Analytical protocols;  
Appendix 9 - Heavy metal and mineral analysis, pesticide content.

Analysis shows (analytical certificate in Appendix 9), that **Zanthin® Extract Astaxanthin Complex** contains no significant amounts of pesticide residues or evidence of inorganic or heavy metal contamination. The manufacturing facility of U.S. Nutra is presently regulated under USFDA cGMP for the manufacture of food.

Consistency between batches is shown in Appendix 10 (Analytical certificates of a selection of representative batches of **Zanthin® Extract Astaxanthin Complex**

## Section 8

### Stability of *Haematococcus* preparations: Algae, Zanthin® Extract Astaxanthin Complex and Capsules

The stability of **Zanthin® Extract Astaxanthin Complex** has been studied over a period of fourteen months (Appendix 11), showing that no significant change in carotenoid levels occurs. To ensure tight control of product stability, ALGATECHNOLOGIES supply their dried algal meal vacuum-sealed in aluminium foil bags, packaged under an oxygen-free atmosphere, to exclude light, air, and moisture, and recommend storage at 5°C or less, as described in Appendix 11. U.S. Nutra plans to sell **Zanthin® Extract Astaxanthin Complex** for use in the manufacture of dietary supplements, U.S. Nutra has therefore also substantiated (Appendix 11) the suitability of their product for encapsulation, and of its stability subsequent to encapsulation. It will be seen that the astaxanthin content of **Zanthin® Extract Astaxanthin Complex** after encapsulation showed no degradation over an 8 month period. Similar data on **Zanthin® Extract**

**Astaxanthin Complex** formulated as a beadlet preparation is supplied Appendices 11a-11c.

## **Section 9**

### **History of the Use of *Haematococcus Pluvialis*, the source of Zanthin® Extract Astaxanthin Complex**

The occurrence of astaxanthin in the food chain is widespread. It occurs in salmonids and crustaceans, which are the major dietary source in humans, although astaxanthin containing dietary supplements are also used. It also occurs in certain yeasts (e.g., *Phaffia* spp), and in certain algae (e.g. *Haematococcus Pluvialis*). Salmon is an especially rich source of astaxanthin in the human diet, containing typically 1-37mg/kg of astaxanthin as shown in Table 5]

**Table 5. Amount of Astaxanthin present in different species (Maher 2000)**

Species	Amount of Astaxanthin present per Kg
Atlantic Salmon	3-11mg
Sockeye Salmon	26-37mg
Rainbow Trout	1-13mg
Yeast ( <i>Phaffia</i> sp.)	30-800mg
Algae ( <i>Haematococcus pluvialis</i> )	10,000-30,000mg

Salmonids obtain their astaxanthin from dietary sources. In the wild state, this is primarily krill, a small crustacean. More recently, with the worldwide increase in fish-farming, farmed salmon have had their feed supplemented with astaxanthin to improve the pink coloration of the flesh. Both synthetically prepared astaxanthin and preparations derived from *Haematococcus* (White et al., 2002) have been used successfully for this purpose. A typical examples of the latter category is NatuRose™ (Cyanotech 1999). Similar products are used in Japan and Canada and in the EU. Astaxanthin has also been approved as a permitted feed additive in salmonids (E161j) in Europe, where, under council directive 70/524 mixtures containing canthaxanthin with astaxanthin are allowed provided that the total concentration of the mixture does not exceed 100mg/kg in the complete food stuff (FSA 2000).

Salmon (wild and farmed) is a well established foodstuff, and as such is regarded as safe, healthy, and is highly recommended by nutritionists (Davidson 1993). These data show that both astaxanthin itself and the *Haematococcus pluvialis* algae from which it is commonly derived in nature, are already an established part

of the human food chain, albeit hitherto indirectly due to the consumption of salmon and crustaceans.

Several companies have been marketing preparations of the astaxanthin-rich *Haematococcus pluvialis* as a human dietary supplement, both in the USA and in other countries. The history of usage in America is supported by the following New Dietary Ingredient Notifications for astaxanthin-related products, which were not objected to by FDA:

1) Micro Gaia Inc. – premarket notification for the extract of *Haematococcus pluvialis* algae filed on March 7, 2002 (Micro Gaia 2002);

2) Aquasearch, Inc. – premarket notification for *Haematococcus pluvialis* algae filed on February 22, 2000 (Aquasearch 2000i)

3) Cyanotech Corp. – premarket notification for *Haematococcus pluvialis* algae filed on March 22, 1999 and May 25, 1999 (RPTs 45 and 50).

## **Section 10**

### **Anticipated levels of Intake and Usage of Zanthin<sup>®</sup> Extract Astaxanthin Complex**

In the UK, a typical 'salmon meal' would consist of a can of 418g Atlantic Salmon, which, using the data in Table 5 would have an average astaxanthin content of approx 3mg of astaxanthin. In parts of the world where sockeye salmon (30mg/kg astaxanthin) is more popular, the same meal (418g sockeye salmon) would contain around 12mg of astaxanthin.

On the basis of these figures, and given the long-established, large-scale and worldwide consumption of various species of salmon by humans, it can reasonably be expected that a single dose of 3-12mg astaxanthin would be safe.

This is supported by the evidence of clinical trials (See Section 11 and Section 13), where no adverse effects have been reported with administration of daily doses of between 2-8mgs of *Haematococcus*-derived astaxanthin and the findings of Davidson (Davidson et al. 1993)

It is the intention of U.S. Nutra to market **Zanthin<sup>®</sup> Extract Astaxanthin Complex** for use in dietary supplement products containing up to a maximum daily intake of 5mgs of astaxanthin, which falls well within the range of astaxanthin levels found in a typical single salmon meal.

Cyanotech and Mera in the US, and Astacarotene in the EU have marketed *Haematococcus* - derived products with similar astaxanthin levels and recommended daily dosage, for consumption by humans on a daily basis. Examples of their products, containing at least 2 – 5mgs per capsule form are:-

1) Aquasearch Inc (now Mera) , market Astafactor soft-gels containing 5mg (Mera 2004i) and 2.5mg (Mera 2004ii) of astaxanthin see also Aquasearch 2000i.

2) Micro Gaia market AstraReal capsules that contain 2mg of astaxanthin based on an extract of *Haematococcus*, currently sold as Astavita™ (Astavita 2004).

3) Purecaps market Cyanotech's BiAstin (*Haematococcus* algal meal ) in 4mg soft-gels capsule (Purecaps 2004)

The intention of U.S. Nutra is to market **Zanthin® Extract Astaxanthin Complex** as dietary supplement ingredient for a daily dose of up to 5mgs of astaxanthin and is therefore in line with the following:-

1) The astaxanthin levels found in other companies' products intended for the same purpose and dose rate.

2) The levels of astaxanthin consumption that could be reasonably assumed to be safe from worldwide consumption of well established foodstuffs

3) The clinical and toxicological data published with regard to safety of *Haematococcus* consumption (see sections 11 and 13 below).

It has already been demonstrated that Zanthin® is phytochemically substantially equivalent to preparations of the dried *Haematococcus* algae, of which it is simply a supercritical carbon dioxide extract. On the basis of this, it maybe concluded that capsules or other oral preparations containing the recommended quantity of astaxanthin from **Zanthin® Extract Astaxanthin Complex** are substantially equivalent to other *Haematococcus*-derived astaxanthin-containing products already on the market.

## **Section 11**

### **Review of Human Safety Data for Astaxanthin-Containing Products**

#### **Human Clinical Trials Safety Report on *Haematococcus pluvialis***

A number of clinical trials of *Haematococcus* have been conducted, by various workers. Examples of such trials by Chew *et al.* (2003 and 2004), Lignell (2001), Mera Pharmaceuticals and Spiller *et al.* (2003) are given below:

#### **"Astaxanthin Stimulates Immune Response in Humans in a Double-blind Study," (Chew et al. 2003 CONFIDENTIAL; Chew et al., 2004)**

(Double-blind placebo-controlled, 0mg / 2mg / 8mg astaxanthin per day for 8 weeks).

#### **Summary:**

42 female human subjects enrolled on a double-blind placebo-controlled study. 14 subjects received 8mg/day dose and 14 subjects received 2mg/day dose, via *Haematococcus pluvialis* algal concentrate meal, and 14 subjects received a placebo; for 8 weeks. Subjects underwent blood examination on weeks 0, 4, & 8, and samples were examined for lymphoproliferation, leukocyte subset, natural killer cell cytotoxic activity and oxidative damage to DNA. Lipid peroxidation was also measured.

Subjects treated with astaxanthin showed a statistically significant ( $P < 0.05$ ) increase in lymphoproliferation, significant ( $P < 0.05$ ) increase in total numbers of T cells and B cells and significantly higher natural killer cell cytotoxic activity. Groups treated with 8mg/day astaxanthin did not show a statistically significant increase in total T cell and B cell numbers compared to the group treated with 2mg/day.

Oxidative damage to DNA was significantly decreased ( $P < 0.01$ ) in groups treated with astaxanthin compared to control group, although groups treated with the higher 8mg/day doses did not show a statistically significant reduction in oxidative damage compared to subjects treated with a 2mg/day dose. Lipid peroxidation was not significantly different for treated groups compared to control groups.

Therefore dietary astaxanthin significantly enhanced immune response, and significantly decreased oxidative DNA damage. No adverse effects due to treatment with astaxanthin were reported in this study.

#### **US Patent 6,245,818 B1** (Lignell 2001)

(4mg/day dose astaxanthin via algal meal capsule, for 6 weeks).

#### **Summary:**

40 healthy human subjects were enrolled on a placebo-controlled trial. 20 received 4mg/day doses of astaxanthin via algal meal capsule, 20 received a placebo, for 6 weeks.

Subjects were tested for changes in explosive strength, endurance strength, and weight. No statistically significant differences were found in any tested parameter. Although not statistically significant, there was a notable increase in improvement for strength/endurance in the astaxanthin group over the placebo group.

No adverse effects were reported.

## **"Astafactor® Technical Report" (Mera 2000)**

228 mg/day of algal meal equivalent to 3.85 mg/day astaxanthin in Low Dose.  
1140 mg/day of algal meal equivalent to 19.25 mg/day astaxanthin in High Dose.

### **Summary:**

33 human volunteers aged between 28 and 62 were given a daily dose of either the Low Dose supplement or High Dose supplement for 29 days. Medical examination of all subjects revealed no observable ill effects and blood and urine examinations from all subjects revealed no toxicity for subjects on either dose.

The algal meal preparation met FDA limits on heavy metal content.

Therefore a daily dose of 5mg astaxanthin via algal meal appears to present no significant safety concerns.

## **"Safety of an Astaxanthin-Rich *Haematococcus pluvialis* Algal Extract: A Randomised Clinical Trial" (Spiller et al 2003)**

2mg astaxanthin via 40mg. *H. pluvialis* algal extract 3 times a day.

### **Summary:**

35 human subjects aged 35-69 enrolled on a randomised, double-blind trial of 8 weeks with a placebo-control group. 19 subjects received 2mg astaxanthin through a 40 mg algal extract, 3 times a day. 16 received a placebo.

Blood pressure and blood chemistry examinations conducted at 0, 4, 8 weeks showed no statistically significant differences between the groups except for serum calcium, total serum protein and eosinophil count. These differences were small enough to have no clinical importance.

This suggests that a 6mg/day dose of astaxanthin taken in the form of algal extract can be safely consumed by humans.

## **Section 12**

### **Microbiological Information on Astaxanthin-rich Oleoresin**

U.S. Nutra's studies on the microbiological load of the oleoresin are contained in Appendix 14. In essence: the use of CO<sub>2</sub> as extraction solvent, and the use of appropriate preservatives both assist the inhibition of food-borne and other microorganisms in the product.

## Section 13

### Safety, Bioavailability and Toxicity Studies on *Haematococcus*-derived Astaxanthin Products

Information used in the following section includes data from peer-reviewed journals and also data derived from research sponsored by the astaxanthin supplement manufacturers.

#### Toxicity Studies

The toxicity of *Haematococcus pluvialis* and its derivatives have been the subject of a number of studies, which are presented in Table 6 below.

**Table 6. Toxicity studies**

SOURCE OF ASTAXANTHIN	AMOUNT OF MATERIAL TESTED	AMOUNT OF ASTAXANTHIN PRESENT	TOXICITY STUDY	REFERENCE
<i>Haematococcus pluvialis</i> dry algal meal contains 2% of total Astaxanthin	50mg/kg of algal meal which corresponds to 3.5g algal meal per 70-kg body weight of an adult man	1mg of total Astaxanthin, or equivalent to a dose of 70mg of total Astaxanthin to an adult man	28 day rat study. Post mortem observation failed to detect any sign of toxicity.	Astafactor Technical Report 1
<i>Haematococcus pluvialis</i> dry algal meal contains 2% of total Astaxanthin	10,400 – 18,000 mg/kg of the algal meal which equates to single doses of 720g to 1.2kg in humans	Information not provided	Single dose acute toxicity study with male and female mice. No abnormalities were observed in post mortem examination. All mice served the trial period.	Koyo Mercantile Company Limited refile, cited in Cyanotech NDIN
<i>Haematococcus pluvialis</i> dry algal meal contains 2% of total Astaxanthin	5g/kg Algal meal	Information not provided	13 day LD <sub>50</sub> acute toxicity study in 3 Cooperative groups in rats, were fed 5g/kg of algal meal. No differences in body weight or abnormalities in post mortem examination suggesting an LD <sub>50</sub> greater than 5000mg/kg	Intl. Research & Devel. Corp. 1989. Cited in Cyanotech NDIN Filing
NatuRose™ <i>H. pluvialis</i> spray dried dark red powder contains 1.5% total Astaxanthin content (70% monoesters, 10% diesters, 5% free Astaxanthin)	5g of algae/kg of rat, corresponds to 350g single dose of algal meal to an adult man of 70kg	Total Astaxanthin equates to 75mg/kg or equivalent to 5.25g dose to an adult man. Implies an LD <sub>50</sub> in man is higher than 5.25g single dose of Total Astaxanthin	13 day Acute oral toxicity study. No visible or post mortem abnormalities were observed.	Cyanotech Technical Report 1.
<i>Haematococcus</i> colour (food additive in Japan)	0 – 0.25% <i>Haematococcus</i> colour.		80 F344 rats were split into 4 groups of 20 and were fed a powder containing 0%, 0.025%, 0.075%, and 0.25% <i>Haematococcus</i> colour, for 13 weeks none of the animals died and there were no exposure related changes in body weight gain or food consumption slight increase in the levels of cholesterol were observed but the differences were slight and not defined as an adverse effect.	Ono et al. (1999).

### **Toxicity of Components of Extracts of *Haematococcus pluvialis***

An exhaustive literature search has not found any evidence of toxic components in *Haematococcus pluvialis*. The algae does, however contain small quantities of canthaxanthin. This compound has been ingested by humans for tanning purposes, at a dose rate of 90mg/day, and has been shown in some individuals, to produce crystalline deposits in the retina. These deposits have, however, been shown to be reversible (Aquaseach 2000i, and Leyon et al 1990).

The content of canthaxanthin in **Zanthin<sup>®</sup> Extract Astaxanthin Complex** is approximately 1% of the astaxanthin content (Appendix 1). With the proposed use and dosage, this would mean a canthaxanthin content of approximately 0.05mg in a daily 5mg dose. Extrapolating from these figures: - in order to absorb a 90mg daily dose of canthaxanthin, an individual would have to swallow 1800 capsules per day of U.S. Nutra's **Zanthin<sup>®</sup> Extract Astaxanthin Complex** product in order to achieve the daily dose level of canthaxanthin which has been shown to cause reversible retinal problems in some individuals. We do not consider that such usage is reasonable or probable, and therefore do not consider the low levels of canthaxanthin in **Zanthin<sup>®</sup> Extract Astaxanthin Complex**-containing products to present a toxicity hazard.

Effects of *cis/trans* isomeric change in **Zanthin<sup>®</sup> Extract Astaxanthin Complex**. The slight changes in the ratio of *cis-trans* isomers of astaxanthin in the extract, versus the dried-algal meal do not appear to have any toxicological significance, given that both isomers occur in the algal meal and the extract. Interconversion of E and Z isomers of astaxanthin occurs readily (Chen *et al.* 1999), so this minor change in the isomeric ratio is not unexpected, as mentioned above in section 5. This is also discussed more fully in Appendix 16.

### **BIOAVAILABILITY & SAFETY STUDIES**

#### **Studies on Bioavailability**

The results of two studies on astaxanthin bioavailability are shown below. One study is on the bioavailability of specifically *Haematococcus*-derived astaxanthin (Lignell 2001) however the other material is of unspecified origin. All three studies suggest that astaxanthin, regardless of isomeric form, is rapidly metabolised over a 24hr period, and there is no indication that a daily intake of up to 5mg of astaxanthin would lead to build-up of this compound in the body.

**Table 7. BIOAVAILABILITY STUDIES – SAFETY STUDIES**

SOURCE OF ASTAXANTHIN	AMOUNT OF MATERIAL TESTED	AMOUNT OF ASTAXANTHIN PRESENT	TOXICOLOGY STUDY	REFERENCE
Not stated		100mg	Transport in the plasma by lipoproteins in a similar way to other carotenoids max levels (1.3+/- 0.1mg/L) were reached after 6.7 hours after administration elimination half life was 21 +/- 11hr.	Osterlie et al. (1999a) cited in Guerin 2002.
Algal meal from <i>Haematococcus</i> (Astacarotene)	100mg Astaxanthin per kg feed in the form of an algal meal	100mg	24 rats were divided into 2 groups. 12 were given feed without algal meal, the other group received the feed containing algal meal. No adverse effects reported.	Lignell (2001) US Patent 6, 245, 818

**Safety Studies Involving Human Clinical Trials**

The data presented below (Table 8) includes the results of five clinical trials, in all of the trials only *Haematococcus*-derived astaxanthin products were used In three of these, the algal meal preparations were used, in the other two, supercritical carbon dioxide extracts were used. It should be noted that one of these (Chew et al 2003, 2004), specifically used U.S. Nutra's **Zanthin® Extract Astaxanthin Complex** for their trials.

**Table 8. SAFETY STUDIES – HUMAN CLINICAL TRIALS**

SOURCE OF ASTAXANTHIN	AMOUNT OF MATERIAL TESTED	AMOUNT OF ASTAXANTHIN PRESENT	STUDY WOULD BE EQUIVALENT TO CO <sub>2</sub> EXTRACT	REFERENCE
Mera Pharmaceuticals <i>H. pluvialis</i> algal meal (contains up to 2% total Astaxanthin) (around 5mg per 250mg of algal meal)	228mg of algal meal	3.85mg	33 human volunteer's daily ingestion for 29 days. Medical examination (urine & blood analysis) did not result in any safety concerns.	Astafactor Technical Report 1.
Mera Pharmaceuticals <i>H. pluvialis</i> algal meal (contains up to 2% total Astaxanthin) (around 5mg per 250mg of algal meal)	1.14g of algal meal	19.25mg of Astaxanthin	33 human volunteer's daily ingestion for 29 days. Medical examination (urine & blood analysis) did not result in any safety concerns.	Astafactor Technical Report 1.
		14.4mg	13 healthy patients were divided into 3 groups and given 3 levels of Astaxanthin daily for 2 weeks. Maximum dose being 14.4mg/day. No ill effects were reported.	Miki (1998) cited in Astafactor Technical Report 1.
Astacartene <i>Haematococcus</i> algal meal	100mg Astaxanthin per kg feed in the form of an algal meal	4mg	20 healthy volunteers were given a capsule containing 4mg of Astaxanthin, against 20 healthy volunteers receiving a placebo. No adverse effects were reported from the study but improvements in endurance test were increased in the Astaxanthin group.	Lignell (2001) US Patent 6, 245, 818
CO <sub>2</sub> extract of <i>Haematococcus</i> Algae	109g Astaxanthin/kg of oleoresin concentrate	2 or 8mgs	An 8 week double blind placebo control trial to investigate the immune boosting effects of Astaxanthin was carried out, with subjects taking 0, 2 or 8mg Astaxanthin capsules, once a day. No adverse effects were reported from the study.	Chew et al 2003; 2004
CO <sub>2</sub> extract of <i>Haematococcus</i> Algae		2mgs of Astaxanthin in each capsule	35 adults, randomized, double blind, placebo controlled trial of 8 weeks duration. Subjects ingested 3, 2mg capsule per day. No adverse effects were reported.	Spiller et al., 2003

In summary, the clinical trials performed with the proposed level of intake of *Haematococcus*-derived astaxanthin on a daily basis have not reported any adverse effects, Most of these clinical trials that have been conducted have used *Haematococcus* derived astaxanthin in levels from 2-8mgs. This level is in line with *Haematococcus*-derived astaxanthin products currently marketed in the USA (see Astavita 2004, Mera 2004i, Mera 2004ii), and is therefore in line with the proposed levels of usage of Zanthin®.

## Section15

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## **Section 16**

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