**ADVISORY COMMITTEE ON NOVEL FOODS AND PROCESSES**

# OPINION ON SUBSTANTIAL EQUIVALENCE OF ASTAXANTHIN-RICH OLEORESIN EXTRACTED FROM *Haematococcus pluvialis* CONSIDERED UNDER ARTICLE 5 OF THE NOVEL FOODS REGULATION

**Applicant** Cyanotech Corporation
73-4460 Queen Kaahumanu Highway
102 Kailua-Kona
HI 96740
USA

**Responsible person** Dr. Gerald R. Cysewski

## Introduction

1. A request was submitted by Cyanotech Corporation to the UK Competent Authority in June 2006 for an opinion on equivalence of an astaxanthin-rich oleoresin obtained from the dried algae biomass of *Haematococcus pluvialis* (BioAstin®) to the existing *H. pluvialis* astaxanthin-rich algal meal (Astaxin™) marketed in the EU by Swedish company Astacarotene AB.

2. Astaxanthin is a xanthophyll (oxygenated) carotenoid, which is found in *Haematococcus pluvialis.* This microalgae is part of the diet of fish and crustaceans (e.g. salmon, shrimps) and is responsible for the pink coloration of their flesh, through the ingestion of astaxanthin.

3. Hard gelatine capsules containing the dried biomass of *H. pluvialis* (Astaxin™) have been sold in the EU by the Swedish company Astacarotene AB[1] prior to 1997. It has also been marketing the whole algal product in bulk form to other EU supplement manufacturers under the name of AstaCarox™.

4. This request addresses substantial equivalence according to the five criteria set out in Article 3(4) of regulation (EC) 258/97: composition, nutritional value, metabolism, intended use and level of undesirable substances contained therein.

[1] Astacarotene AB is the new name of the company Astacarotene now owned by Fuji Chemical Industry Co., of Japan.

## Evaluation

## Composition

5. The algal strain cultivated by the applicant to produce *Haematococcus* algae meal is *Haematococcus pluvialis* Flotow strain Steptoe. This strain, which is also known as H2B, has been maintained in pure laboratory culture following its isolation in the Steptoe watershed of Nevada (USA). The applicant states that although the exact strain of *H. pluvialis* used by Astacarotene AB is a trade secret, the known *H. pluvialis* strains are expected to be comparable phytochemically if cultivated in a similar manner.

6. The oleoresin will be produced in two standardised forms with a 5% and 10% astaxanthin content (BioAstin® SCE5 and BioAstin® SCE10). The applicant produces its oleoresin from dried *H. pluvialis* using a supercritical $CO_2$ process to remove the lipid fraction, including carotenoids. The applicant indicates that his extraction procedure is similar to the one used by US Nutra[2] for the production of its astaxanthin-rich oleoresin (Zanthin®) which was given a positive opinion from the UK Competent Authority in accordance with Article 3(4) of the novel food regulation, in June 2004[3].

7. The oleoresin is a thick liquid and contains approximately between 8 and 12% astaxanthin. A series of lots of the raw oleoresin are blended in a stainless steel vat to produce a product with a guaranteed minimum 10% astaxanthin (Bioastin® SCE10). High oleic safflower oil is blended with the raw oleoresin to produce a 5% astaxanthin oleoresin (Bioastin® SCE5).

8. The applicant has compared the composition of the oleoresin with the *H. pluvialis* meal source. The applicant is of the view that the fatty acid composition of the two products is similar. The oleoresin with 5% astaxanthin shows a higher level of oleic acid due to the use of high oleic safflower oil in its manufacturing.

9. The applicant has provided compositional data to show that the fatty acid levels and carotenoid composition are similar in their oleoresin, the dried *H. pluvialis* meal from which it is extracted and the existing *H. pluvialis* algal meal marketed by Astacarotene AB (Astaxin™ and AstaCarox™). Whilst there are small differences in the levels of specific fatty acids, the applicant is of the view that this may be due to variability between *H. pluvialis* strains and/or some analytical variability between labs conducting the analyses, and also the extent to which unsaturated fatty acids have been converted to saturated fatty acids within algal cells during cultivation.

10. The total astaxanthin content in *H. pluvialis* meal and the astaxanthin rich oleoresins represents 2-4% and 5-11% of the total weight, respectively. As

[2] US Nutra is now called Valensa, Inc.
[3] UK Competent Authority opinion on substantial equivalence for US Nutra's astaxanthin-rich oleoresin from *H.pluvialis*, see: http://www.food.gov.uk/multimedia/pdfs/astaxanthinfinal.PDF

is the case for the existing product, E-astaxanthin is the dominant geometrical isomer in the *H. pluvialis* meal used to produce the oleoresin, with a small amount of 9Z and 13Z astaxanthin isomers.

11. The applicant recommends a one-year shelf life providing the oleoresin is stored in the dark at <8°C. HPLC measurements revealed losses of 5% or less during the first six months of storage. Typically a supplement manufacturer would incorporate the oleoresin into gelatine capsules or beadlets soon after receiving shipment. The applicant has also therefore conducted stability studies on soft gelatine capsules and microencapsulated gelatine tablet-grade beadlets containing the oleoresin, which showed both products were stable at room temperature for nine and ten months.

**Discussion:** *The Committee was satisfied that the data comparing the oleoresin, its algal meal source and the existing algal meal show that they are similar in composition and that levels of astaxanthin are comparable.*

**b) c) Nutritional Value and Metabolism**

12. The nutritional value of the oleoresin lies in its carotenoid content, particularly astaxanthin which is a known antioxidant. The applicant highlighted that astaxanthin is an occasional component in the human diet due to its presence in fish and crustaceans. The oleoresin will be an alternative source of astaxanthin and it is not intended to prevent, cure, treat or mitigate any disease or specific condition.

13. The applicant refers to various studies demonstrating the metabolism of astaxanthin. A study by Showalter *et al* (2004) [4] has suggested a greater bioavailability in mice of esterified astaxanthin, the predominant form present in *Haematococcus*, than free astaxanthin. In a human study by Osterlie *et al* (2000)[5], astaxanthin was found to be present in all lipoprotein fractions, after ingestion of a meal containing 100 mg of synthetic free astaxanthin. In another human study by Odeberg *et al* (2003) [6], the oral bioavailability of esterified astaxanthin administered as *Haematococcus* algae meal was shown to be enhanced in human volunteers by the incorporation of the astaxanthin into lipid based formulations, suggesting that dissolution from the matrix and/or incorporation into mixed micelles may limit the rate of bioabsorption.

[4] Showalter *et al* (2004). Plasma appearance and scar tissue accumulation of non-esterified, free astaxanthin in C57BL/6 mice after oral dosing of a disodium disuccinate diester of astaxanthin (Heptax™). Comparative Biochemistry and Physiology Part C 137: 227-236.

[5] Osterlie *et al* (2000). Plasma appearance and distribution of astaxanthin E/Z and R/S isomers in plasma lipoproteins of men after single dose administration of astaxanthin. Journal of Nutritional Biochemistry 11: 482-490

[6] Odeberg, *et al* (2003). Oral Bioavailability of the antioxidant astaxanthin in humans is enhanced by incorporation of lipid based formulations. European Journal of Pharmaceutical Sciences 19: 299-304.

14. In response to a request by the Committee, the applicant provided further information on the proportion of free and esterified astaxanthin present in the oleoresin compared with the algal meal and how different the effectiveness of these two forms of astaxanthin is in the oleoresin. The applicant explained that the *H. pluvialis* meal contains less than 6% free astaxanthin with the remaining present as esters. This is also the case for the existing *Haematococcus* meal (Astaxin™). The applicant presented chromatographic results on the carotenoid fraction of its algal source in its dossier which showed that it contained 70% monoesters of astaxanthin, 10% diesters of astaxanthin and 5% free astaxanthin, with the remainder consisting of beta-carotene, cantaxanthin and lutein. The applicant also provided additional results of HPLC analyses of the existing algal meal and the oleoresin which demonstrated that the ratio of free astaxanthin to total astaxanthin (free and esterified) is similar.

15. Regarding the metabolism of esterified and free astaxanthin, the applicant explained that the consumption of esterified astaxanthin and free astaxanthin results in only free astaxanthin being present in the plasma; however the rate of accumulation in the plasma is slower with astaxanthin esters because hydrolysis of the esters must first take place prior to absorption. The applicant also highlighted that the bioavailability of astaxanthin esters is enhanced when they are formulated into a lipid-based product. The applicant therefore recommends supplement formulators to use a suitable oil carrier for products containing the oleoresin.

**Discussion:** *The Committee was content with information provided on the nutritional value of the oleoresin. The Committee also accepted the additional information provided by the applicant which indicated that the oleoresin and the existing algal meal contained the same ratio of free and esterified astaxanthin with the latter having a slower metabolic rate.*

*Members agreed that the studies provided by the applicant in relation to the efficacy of the novel ingredient were not relevant to the determination of equivalence.*

**d) Intended Use**

16. The applicant intends to market the oleoresin as an ingredient to be used by food supplement manufacturers in hard and soft gelatine capsules and tablets, with an astaxanthin content of no more than 4mg per capsule. This is equivalent to the astaxanthin level found in algal meal food supplements currently found on the EU market such as Astaxin™.

17. The extract will be available at two standardised astaxanthin titers namely: 10% (BioAstin®SCE10) and 5% (BioAstin®SCE5).

***Discussion:*** *The Committee was content that the intended use of the oleoresin as an ingredient in food supplement and the proposed maximum*

*astaxanthin level of 4mg per capsule were equivalent to those of the existing product.*

**e) Levels of undesirable substances**

18. The applicant stated that the oleoresin complies with strict limits for levels of microbiological and heavy metal contaminants and it is free from chemical pesticides. Analyses of the oleoresin have demonstrated that both its heavy metal and microbial contents are within the stated safety limits.

19. The applicant has compared the levels of heavy metals and microorganims in the oleoresin with those found in the existing *H. pluvialis* meal (AstaCarox™), and also in US Nutra's astaxanthin-rich oleoresin (Zanthin®). The heavy metal levels are similar except for the level of arsenic[7] (1.2 ppm) in the oleoresin, which is higher than that found in Zanthin® (<0.5ppm) and AstaCarox™ (<0.05ppm). The levels of microorganisms present in the oleoresin are similar to those for US §'s oleoresin (Zanthin®).

20. The applicant states that the manufacture of the oleoresin complies with quality control standards. Precautions are taken to assure that the manufacturing procedures do not contribute any contaminants and analyses of the algal culture and the final extracts are performed on a contractual basis. In response to a request from the Committee, the applicant provided additional information on the quality assurance procedures indicating that a HACCP plan was in place which includes checking for contamination of *Haematococcus* cultures at every stage of the production process. Cultures are monitored daily and if contaminated, the pond culture is destroyed and the pond liner is sterilised with hypochlorite.

21. The applicant was asked to provide further information on the culture conditions of *H. pluvialis*, additional data to demonstrate the absence of undesirable contaminants (such as cyanobacteria) in the culture systems (closed culture and and open pond culture) and whether seasonal variations in the levels of other undesirable substances in the open ponds were taken into consideration. The applicant indicated that manufacture takes place in Hawaii, where the climate allows relatively constant culture conditions to be maintained in the open pond culture systems. The culture temperatures are also maintained well below ambient temperature through the use of cold deep-sea water which would reduce the likelihood of cyanobacterial contamination. No cyanobacterial contamination of open culture ponds has ever been observed by the applicant, and due to the low culture temperature, low nutrient concentrations and high *Haematococcus* biomass, the applicant is of the view that this is unlikely to occur. In

[7] There is no EU limit on arsenic. In the UK, the Arsenic in Food Regulations (SI 159 no 831) as amended lay down a general limit of 1 mg/kg for total arsenic in food. This Regulation excludes fish and edible seaweed where arsenic is present naturally.

addition, the applicant is of the view that the physical layout and location of the facility make air- or water-borne contamination by microorganisms unlikely. The applicant also asked an independent laboratory to analyse three batches of the oleoresin and three batches of *Haematococcus* algal meal for cyanobacterial toxins, in particular Anatoxin-a and Cylindrospermopsin using ESI/MS and MS/MS. These analyses confirmed that the levels of Anatoxin-a and Cylindrospermopsin are below the limit of detection (0.2g/l) in both the oleoresin and the *Haematococcus* algal meal.

22. *Haematococcus* algae also contain small quantities of canthaxanthin, a related carotenoid which, when ingested in large doses over a long period of time, may crystallise in the retina. The applicant has reported that these reversible retinal inclusions only develop when the cantaxanthin dose is greater that 0.2 mg/kg body weight/day. However, the applicant considers that the levels of canthaxanthin present in the oleoresin are not of any safety concern as a 70kg individual would have to consume more that 100 capsules containing the oleoresin per day to reach this dose. However whilst no data have been provided to support this calculation the levels of cantaxanthin present is lower than that reported for the previously-approved product from US Nutra.

**Discussion:** *The Committee was content that the applicant had quality control procedures in place to minimise the risk of contamination of the algal culture and the oleoresin and noted that batches of the oleoresin had been recently tested and found to contain no detectable levels of cyanobacterial toxins. The Committee expressed some concern that water temperature, physical layout and location of the culture facility were considered by the applicant to be the major factors in preventing contamination with other microbes. In view of this the Committee considered that the applicant should implement a regime to ensure that the final product is tested periodically to confirm the effectiveness of the production controls.*

**f) Additional information**

23. **Labelling:** The applicant intends that the final products will comply with EU legislation for food supplements and provided example labels for the oleoresin.

24. **Safety studies:** The applicant refers to different animal studies to show the safety of the extract. The conclusions from these studies were that the extract exhibits no ill effects on animals. A 4-week human study by Shimada *et al* (2004)[8] demonstrated that astaxanthin could be consumed safely at dosages of 2-12mg per day with no ill effects.

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

25. The applicant highlights that the oleoresin was approved in the USA and launched in 1999 and is currently marketed for use in dietary supplements in at least 20 other non-EU countries. The applicant indicated that there have been no reports of adverse reactions to any food supplements containing the oleoresin, with a recommended astaxanthin dosage of 2-12g/day, for the past 10 years.

**Discussion:** *The Committee was content that the applicant will adhere to EU legislation for labelling of food supplements when labelling the oleoresin.*

## Conclusion

26. The Committee concluded that Cyanotech Corporation has demonstrated the equivalence of their astaxanthin-rich oleoresin obtained from *H. pluvialis* with the existing astaxanthin-rich *H. Pluvialis* meal according to the criteria set out in Article 3(4) of the Novel Foods Regulation (EC) 258/97. The Committee recommended that the applicant should implement a regime to ensure that the final product is tested periodically to confirm the effectiveness of the production controls in preventing contamination with toxigenic bacteria.

27. This opinion applies solely to the oleoresin as an ingredient to be used by food supplement manufacturers in hard and soft gelatine capsules and tablets, with an astaxanthin content of no more than 4mg per capsule.

28. Therefore, the astaxanthin-rich oleoresin produced by Cyanotech Corporation can be considered to be substantially equivalent to the existing astaxanthin-rich meal produced by Astacarotene AB.

**February 2007**