

ADVISORY COMMITTEE ON NOVEL FOODS AND PROCESSES

ASTAXANTHIN-RICH EXTRACT FROM *Haematococcus pluvialis* ALGAE

Issue

The Committee is asked to consider the information provided for an astaxanthin-rich oleoresin extracted from the algae *Haematococcus pluvialis* using solvent extraction. The manufacturer of this product, Parry Nutraceuticals, requests the opinion of the UK Competent Authority (CA) that this oleoresin should be considered substantially equivalent to an existing product.

Background

1. Astaxanthin is a xanthophyll (oxygenated) carotenoid, which is found in *H. 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. Astaxanthin, unlike some carotenoids, does not convert to Vitamin A (retinol) in the human body. However, it is a powerful antioxidant with perceived functional benefits if consumed as a food supplement.
2. Regulation (EC) 258/97 makes provision for novel foods or ingredients that are substantially equivalent to an existing product to be placed on the market once the applicant has notified the Commission. In all cases to date, the Commission has required that the applicant first obtain an opinion on equivalence from a Member State. Parry Nutraceuticals Ltd is requesting such an opinion from the UK Competent Authority.
3. According to Article 3(4) of (EC) 258/97, the notification procedures applies to “foods or food ingredients...which on the basis of the scientific evidence available and generally recognised or on the basis of an opinion delivered by one of the competent bodies...are substantially equivalent to existing foods or food ingredients as regards their:
 - Composition
 - Nutritional value
 - Metabolism

- Intended use, and
 - level of undesirable substances contained therein”.
4. This is the fourth request that the UK has received for an opinion on the equivalence of an astaxanthin-rich oleoresin. Parry Nutraceuticals currently market a *H. pluvialis* algae cell powder rich in astaxanthin, which they export to a number of countries, including EU Member States. The applicant seeks an opinion on an astaxanthin-rich oleoresin obtained from the cell powder for use in food supplements. This is consistent with the three previous requests for astaxanthin-rich extracts.
 5. The first UK opinion was issued in 2004 to Valensa (US Nutra). In 2007, a second opinion was issued for a similar astaxanthin-rich extract, (again from *H. pluvialis*) to Cyanotech Corporation. In both cases the Committee agreed that the extracts were substantially equivalent to an astaxanthin-rich algal meal which had been on the EU market in food supplement form since 1995. A third UK opinion was issued in 2008 to Algatechnologies (1998) Ltd, who had established equivalence to Valensa’s oleoresin product.
 6. Parry Nutraceuticals have provided data which in their view demonstrates that their oleoresin is equivalent to the extract marketed by Cyanotech. This view is substantiated by a comparison of the composition of key constituents in both the powder and oleoresin forms.
 7. In previous applications the case for equivalence was made largely on the basis that the oleoresin contained a comparable amount of astaxanthin and other carotenoids when compared with the original algal meal product, and it was accepted that there would be differences in the overall gross composition of the meal compared with an extracted oleoresin, and many of the components in the meal would be either absent from the extract or present in reduced proportions. Although not clearly explained in the dossier, Parry Nutraceuticals have used a different approach by providing compositional data to show that both their starting material (the cell powder) is equivalent to Cyanotech’s starting material and that the lipid and carotenoid composition of the resulting oleoresins are not substantively different.
 8. Under Article 3(4) of the Novel Foods Regulation (EC) 258/97, the applicant is requesting an opinion from the UK Competent Authority on equivalence of its astaxanthin-rich oleoresin from *H. pluvialis* compared with Cyanotech’s product, which in turn was demonstrably equivalent to an algal meal (i.e. cell powder) product on the market in the EU before the entry into force of (EC) 258/97. Parry Nutraceuticals use a different extraction solvent in their process so in order to ensure a meaningful comparison, the Secretariat has summarised the compositional data for both the cell powder and the oleoresin. The application

dossier and appendices are attached at Annex A; additional information is attached at Annex B.

9. The applicant intends to market their oleoresin in three forms (2.5, 5.0 and 7.0%), which in the dossier is referred to as **AstaNatural** and Cyanotech's equivalent product is called **BioAstin**). For ease of reference, this paper relates to the starting materials and the end products as cell powder and oleoresin respectively.
10. A non-confidential version of the application dossier will be placed on the FSA website to allow the public to input into the UK assessment and comments received after the ACNFP meeting will be forwarded to Members for consideration.

Evaluation

a) Composition

Annex A, p. 4 – 20 and Appendix 1

11. The applicant cultivates *H. pluvialis* algae in open raceway ponds with astaxanthin synthesis being induced by nutrient limitation and sunlight. On completion of astaxanthin synthesis, the cells are harvested by filtration and contaminants removed by washing before the cell biomass is spray dried.
12. Astaxanthin is extracted from the cell powder using food grade ethyl acetate. The extracted oleoresin is separated from the spent powder by centrifugation. The oleoresin is then subjected to a three stage distillation process under vacuum conditions to remove the residual solvent. The oleoresin is then diluted, as required, using olive oil to the specified concentration. (Annex A, Appendix 2).
13. The applicant has carried out a comparison of the proximate composition of Cyanotech's and their own *H. pluvialis* cell powder. (Annex A, Appendix 10). The applicant has sought to summarise these data in Table 5 (Annex A, p 16) however the Secretariat notes that the mean figures do not appear to reflect those seen in Appendix 10, and are not consistent with the specification described in Appendix 9A. An amended version of Table 5, produced by the Secretariat comparing the applicant's cell powder with that of Cyanotech's is presented below and the applicant has been contacted for an explanation. The Secretariat notes that the observed differences in the cell powder could be due to slightly different growth conditions used by the two manufacturers.

Parameter (%) w/w	Parry Cell Powder (a)	Cyanotech Cell Powder (b)
Protein	26.68 ±0.14	28.6 ±1.30
Carbohydrate	32.83 ±0.84	44.8 ±3.30
Lipid	10.86 ±0.69	13.1 ±0.10
Minerals	8.77 ±0.09	19.2 ±1.10
Moisture	4.88 ±0.04	6.8 ±1.00

Total Astaxanthin	1.52 ±0.01	2.58 ±0.10
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(a) Values are the mean ±SD of 3 batches

(b) Values are the mean ±SD of 3 batches, as reported by Cyanotech in their 2006 application

14. The applicant has also included a comparison of the proximate composition of its 7% oleoresin and Cyanotech's 10% product. (Annex A, Table 8, p18; Appendix 3A and Appendix 4). The oleoresins are obtained from the respective cell powder by extraction with ethyl acetate (Parry) and supercritical carbon dioxide (Cyanotech). The results are summarised in the table below.

Parameter (% w/w)	Parry 7% Oleoresin (a)	Cyanotech 10% Oleoresin (b)
Protein	0.91 ±0.02	3.3 ±1.10
Carbohydrate	0.10 ±0.01	47.3 ±5.50
Lipid	94.95 ±0.18	42.8 ±0.60
Ash	3.79 ±0.19	1.2 ±1.40
Moisture	0.21 ±0.04	5.5 ±2.50
Total Astaxanthin	7.22 ±0.10	10.57 ±0.43

(a) Values are the mean ±SD of 3 batches (Secretariat to check with applicant)

(b) Values are the mean ±SD of 2 batches, as reported by Cyanotech in their 2006 application

Fatty Acids

15. The applicant has included a comparison of the fatty acid profile of their algal cell powder with Cyanotech's cell powder. (Annex A, Table 6, p16). The applicant states that the fatty acid profile is comparable to Cyanotech's and that the slight variation in values could be due to the mode of cultivation and medium used.

16. The applicant has also included a comparison of the fatty acid profile of their 2.5, 5.0 and 7% oleoresins with the Cyanotech 5 and 10% oleoresins. (Annex A, Table 4, p14 and Table 9, p 19).

Carotenoid Profile

17. The applicant has compared the carotenoid profile of Parry Nutraceutical's and Cyanotech's cell powder. (Annex A, Table 7, p17). See table below.

Carotenoids (% w/w)	Parry Cell Powder (a)	Cyanotech Cell Powder (b)
Betacarotene	0.01 ±0.01	0.06 ±0.01
Canthaxanthin	0.02 ±0.01	0.05 ±0.01
Astacene	0.05 ±0.01	NA
Semiastacene	0.02 ±0.01	NA
Dicis astaxanthin	0.02 ±0.01	0.03 ±0.01
Trans astaxanthin	1.15 ±0.12	1.94 ±0.13
9 cis astaxanthin	0.14 ±0.02	0.35 ±0.02

Carotenoids (%) w/w	Parry Cell Powder (a)	Cyanotech Cell Powder (b)
13 cis astaxanthin	0.09 ±0.01	0.25 ±0.05
15 cis astaxanthin	NA	0.02 ±0.01
Lutein	0.03 ±0.02	0.13 ±0.02
Total carotenoids	1.52	2.83

(a) Values are the mean ±SD of 3 batches. (Secretariat to check with applicant)

(b) Values are the mean ±SD of 3 batches, as reported by Cyanotech in their 2006 application

NA: Not available

18. The applicant has also compared the carotenoid profile of their 5% and 7% oleoresin with the Cyanotech oleoresins. (Annex A, Table 10, p20). This is summarised in the table below.

Carotenoids (%) w/w	Parry 5% Oleoresin (a)	Parry 7% Oleoresin (a)	Cyanotech 5% Oleoresin (b)	Cyanotech 10% Oleoresin (b)
Betacarotene	0.03 ±0.01	0.04 ±0.01	0.13 ±0.02	0.26 ±0.07
Canthaxanthin	0.06 ±0.02	0.08 ±0.02	0.14 ±0.04	0.15 ±0.05
Astacene	0.16 ±0.03	0.22 ±0.05	NA	NA
Semiastacene	0.07 ±0.02	0.10 ±0.02	NA	NA
Dicis astaxanthin	0.05 ±0.01	0.08 ±0.02	0.07 ±0.02	0.19 ±0.14
Trans astaxanthin	3.86 ±0.22	5.39 ±0.35	3.71 ±0.02	7.02 ±0.60
9-cis astaxanthin	0.47 ±0.10	0.69 ±0.15	1.0 ±0.13	1.95 ±0.44
13-cis astaxanthin	0.31 ±0.08	0.48 ±0.10	0.46 ±0.03	1.22 ±0.57
15-cis astaxanthin	NA	NA	0.04 ±0.01	0.06
Lutein	0.09 ±0.02	0.12 ±0.03	0.22 ±0.02	0.18 ±0.10

(a) Values are the mean ±SD of 3 batches. (Secretariat to check with applicant)

(b) Values are the mean ±SD of 3 batches (5% oleoresin) or 4 batches (10% oleoresin), as reported by Cyanotech in their 2006 application

NA: Not available

Composition Differences

19. There are some minor compositional differences seen in both the comparison of cell powders and the oleoresins. The applicant views these to be expected and due to the use of different strains of *H. pluvialis* and differing culture conditions. Although both companies employ a system involving open ponds, the Cyanotech system uses open ponds only in the final stages of growth. Whilst the different extraction solvents used will also contribute to any differences in the oleoresins, given that previous opinions have been issued on the basis of comparison of oleoresin with cell powder, the Secretariat suggests that any differences as a result of the solvent extraction process are important only if they give rise to differences in the level of undesirable substances.

b), c) Nutritional Value and Metabolism

Annex A, p. 21 - 25

20. The applicant is of the view that there would be no difference in the metabolism of their product compared with its existing counterpart because of the similarities in the carotenoid profile. See tables above, paragraphs 16 and 17.
21. The applicant has included a number of bioavailability studies relating to the uptake and metabolism of astaxanthin in rainbow trout, Atlantic salmon and humans. In a human study by Odeberg *et al.*, (2003), it was shown that the oral bioavailability of astaxanthin is enhanced by the incorporation of a lipid based formulation.
22. The applicant provides data showing that the oleoresin is stable for a minimum period of one year and has included stability data for the three formulations. (Annex A, Appendix 11).

d) Intended Use

Annex A, p. 26

23. The applicant intends to market its astaxanthin oleoresin in the EU for use in food supplements. The oleoresin product will be available in three different concentrations (2.5, 5.0 and 7.0%).
24. The applicant intends to produce softgel capsules for use as food supplements containing up to 4mg of astaxanthin. This dose level is in line with astaxanthin levels found in similar products on the market.

e) Levels of Undesirable Substances

Annex A, p. 26 - 38

Chemical Contamination

25. The applicant's QC system regularly checks both the cell powder and oleoresin for heavy metals, pesticides and production contaminants. The applicant has provided results from three separate batches of each formulation that have been carried out in accredited laboratories (Annex A, Appendix 3A). All were shown to be below detectable levels.

Microbial Contamination

26. The applicant routinely tests batches of the oleoresin for microbiological contamination as part of their HACCP quality control system. Analyses include standard plate count, detection of yeasts and moulds and coliforms, *E. coli*, *Salmonella* and *Staphylococcus*. The applicant has included test reports for three samples of each formulation in Annex A, Appendix 13. These reports show all microbial contaminants were below acceptable levels.

Solvent Residues

27. Ethyl acetate is permitted to be used by Directive 88/344/EC as an extraction solvent in the processing of food ingredients in compliance with good manufacturing practice. The applicant states that the solvent is removed using a vacuum to ensure the residual solvent level is kept below the specified limit of 100ppm. The information provided in Appendix 3A shows residual levels of hexane only rather than ethyl acetate. Additional data on ethyl acetate are provided in Annex B. The client also states that the levels of residual solvent in the astaxanthin oleoresin are continually monitored and is designated a critical control point in the HACCP plan.

g) Additional Information

Annex A, p. 38 - 39

28. The applicant states that they have ISO 9000 (2000), cGMP, HACCP and ISO 14000 accreditation and that stringent hygiene and quality control systems are used throughout its production facility. (Annex A, p38). However, no certificates have been forwarded to confirm this statement.

29. The applicant has not provided any information on the product label.

Committee Action Required

30. The Committee is asked if it has any objections or comments to raise and whether it agrees that substantial equivalence has been established between Parry Nutraceutical's astaxanthin-rich extract derived from *H. pluvialis* and an existing product, also produced from *H. pluvialis*, in accordance with Article 3(4) of Regulation (EC) 258/97.

31. If not, the Committee is asked what additional information the applicant should supply in order to demonstrate equivalence.

**Secretariat
January 2009**

Annexes attached:

Annex A: Application dossier and appendices

Annex B: Additional information on ethyl acetate

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**Application dossier for astaxanthin-rich extract from
Haematococcus pluvialis algae**

**Secretariat
February 2009**

ADVISORY COMMITTEE ON NOVEL FOODS AND PROCESSES

Additional information on ethyl acetate

**Secretariat
February 2009**