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

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INTRODUCTION

1. A request was submitted by Algatechnologies (1998) to the UK Competent Authority in June 2007 for an opinion on equivalence of an astaxanthin-rich oleoresin obtained from *Haematococcus pluvialis* algae, using super critical carbon dioxide (CO_2) extraction, to the existing *H. pluvialis* astaxanthin-rich oleoresin marketed in the EU by Valensa Inc. (US Nutra).

2. Algatechnologies (1998) originally supplied Valensa with *H. pluvialis* meal as a raw material but now seeks clearance to manufacture astaxanthin-rich oleoresin at a European plant using the same CO_2 technology and using the same source material, and to market this as a novel ingredient in the EU.

3. The product manufactured by Valensa is already authorised as a novel ingredient in the EU, following an assessment by the UK authorities and notification made to the European Commission in June 2004, according to Article 5 of the novel foods regulation (EC) 258/97.

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.

EVALUATION

(a) Composition

5. The applicant cultivates *H. pluvialis* using a closed tubular system for producing enriched cells that provide a high concentration of astaxanthin in the algal biomass. The two-stage process uses optimised growth conditions and strain selection which produces a fine, free flowing red flake meal.

6. The applicant produces its extract from the *H. pluvialis* algal biomass using supercritical CO_2 extraction. A similar extraction process using CO_2 is used in the manufacture of the existing product.

7. The applicant has provided the following specification for their oleoresin:

Specification	
Appearance	Dark red viscous oleoresin
Astaxanthin complex	Min. 10 % (Spectrophotometric Method)
Moisture	≤ 5% (Karl Fischer)
Solubility	Lipid soluble
Heavy metals	≤ 10 ppm as lead
Microbiological Data:	
Total plate count	< 1000 cfu/g
Yeast and moulds	< 100 cfu/g
E. coli	Absent
Salmonella	Absent
P. aeruginosa	Absent

8. The applicant has provided a chemical comparison of their oleoresin with the existing product. The relevant data are summarised in the table below.

Carotenoid profiles of two batches of the Novel Ingredient compared with two batches of the existing product (%w/w of the product)				
	Existing Product (Valensa)		**Novel Ingredient (Algatechnologies)**	
E-astaxanthin	8.35	8.10	7.58	8.25
9Z-astaxanthin	1.05	1.13	1.73	1.33
13Z-astaxanthin	0.51	0.68	0.70	0.42
15Z-astaxanthin	0.19	0.19	0.10	0.09
diZ astaxanthin	ND	ND	ND	ND
Total astaxanthin	**10.05**	**10.01**	**10.08**	**10.04**
Free astaxanthin	0.22	0.24	0.12	0.18
Mono-esters	8.66	8.78	8.11	8.49
Di-esters	1.22	1.08	1.87	1.42
Beta-carotene	0.01	0.02	0.01	0.01
Canthaxanthin	0.05	0.05	0.02	0.04
Lutein	0.07	0.09	0.08	0.09
Zeaxanthin	0.00	0.01	0.01	0.01
Violaxanthin	0.01	0.02	0.01	0.01
Total other carotenoids	**0.15**	**0.19**	**0.12**	**0.16**
Total carotenoids	**10.20**	**10.20**	**10.20**	**10.20**
Batch	**EXT050809**	**EXT110524**	**EXT060201**	**EXT060211**

Carotenoid profile

9. The applicant has compared the carotenoid profile of the NI with the existing (Valensa) product. This is summarised in the table above and in the

application dossier. The carotenoids were determined by HPLC with UV/visible detection at 472 nm, with and without hydrolysis of esters. The data in the table indicate that the existing product and the NI have a highly comparable composition.

10. In both products, majority of astaxanthin was present as the mono-esterified form, with lesser amounts as the di-ester and minimal amounts of free astaxanthin. All samples showed similar profiles of the minor carotenoids present (beta-carotene, canthaxanthin, lutein). The applicant comments that absolute concentrations of total carotenoids would not differ between the two extraction processes because both extracts are adjusted with olive oil to give 10.2% astaxanthin complex (total carotenoids) which will deliver at least 10% pure astaxanthin in the final commercial product.

11. The E-isomer of astaxanthin was dominant in all samples although extracts showed a slight shift towards the Z-form. The applicant comments that shifts between geometric isomers are known to happen as a result of environmental conditions (e.g. temperature).

12. The applicant is of the view that the small differences in ratios of Z- and E-isomers, esterification and other carotenoids do not appear to be systematic, and differences between the two extraction process were not statistically significant at the $p<0.05$ level (paired 2-sample t-test). Small variations in the ratios of Z- and E- isomers in samples of the *H. pluvialis* biomass were thought to relate to batch to batch variations in production systems and growing conditions.

Fatty acids

13. Fatty acid analyses of the NI and its existing counterpart are detailed in Table 2 and Appendix 4 of the applicant's dossier. The predominant fatty acid present in all samples was C18 (overall average 69%) followed by C16 (20%) and C20 (7%). Ratios of fatty acids varied between extracts but this was to be expected due to relative standard deviations of up to 38% for the principal fatty acids. The applicant concludes that were no significant differences in total C16, total C18 or total C20 levels at the $p<0.05$ level (paired 2-sample t-test). Differences, where present were due to natural variations and analytical uncertainties.

Other constituents

14. Results of analysis for protein, moisture, carbohydrate, total fat and ash are provided in Table 3, 4 & 5 of the application and Appendix 6-8. No differences between the extract NI and its existing counterpart were observed for protein, fat, carbohydrate or ash content. Some small differences were observed for moisture content (1.3-4.0%).

Discussion: *The Committee noted that the number of batches tested was too small to perform meaningful statistical analysis but the results did not indicate any substantive differences in composition and that the levels of astaxanthin and other carotenoids are comparable.*

(b), (c) Nutritional Value and Metabolism

15. The applicant comments that the carotenoid profile of their oleoresin is indistinguishable from that of the Valensa product which was considered to correspond closely to the levels of carotenoids in the existing algal meal. No differences in nutritional and metabolism value were expected.

Discussion: *The Committee was content with information provided on the nutritional value and metabolism of the oleoresin.*

(d) Intended Use

16. The applicant intends to market the NI to dietary supplement manufacturers who will then dilute the product in a suitable carrier (e.g. olive oil) to produce capsules containing up to 4 mg of astaxanthin. This dose level is in line with astaxanthin levels found in existing similar products.

17. The applicant intends to communicate the dose level to supplement manufacturers in the Manufacturers Safety Data Sheet which states that the supplement is for consumption in capsules, tablets, etc with a maximum dose of 40 mg oleoresin per day, which is equivalent to 4 mg of astaxanthin.

Discussion*: The Committee noted 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

Trace elements

18. The applicant has provided results of the analysis for 31 trace elements using inductively coupled plasma (ICP) in two batches of product from each extractor. The applicant notes that because the CO_2 extraction method is designed to concentrate lipophilic compounds, levels of all electrolytes are relatively low.

19. The applicant states that differences in trace elements were not statistically significant at the $p<0.05$ level (paired two-sample t-test). The applicant suggests that any differences in trace elements cannot be due to differences in nutrient composition of the production system because these are closely controlled; however the quality of the process water is not completely constant and varies between certain permitted limits. The applicant also states that most of the cations and some anions are actively accumulated in the algal cells, sometimes to concentrations a few hundred-fold higher than in the medium. Minor changes in the ion concentrations in the growth medium can cause large differences in concentrations in the cells.

20. The applicant also provided results of ICP analysis of 3 batches of the algal biomass which is used as the source material for preparation of the oleoresin. A degree of batch-to-batch variability was observed for certain elements, which the applicant attributes to variation in the algal biomass.

Diphenylamine contamination

21. The applicant provided results of gas chromatograph screening for pesticide residues in four batches of product. The screen is capable of detecting up to 198 different pesticide residues, including diphenylamine (DPA), with limits of detection (LOD) of 0.005-0.05 mg/kg and limits of quantification (LOQ) of 0.01-0.2 mg/kg depending on the sensitivity of the GC detectors to the different compounds and on the sample matrix. No residues were detected.

22. During the public consultation a question was raised regarding the possible low-level presence of diphenylamine in batches of the *Haematococcus* algal meal. The applicant conducted further analyses of oleoresin and biomass samples for DPA using LC/MS/MS capable of detecting extremely low levels of DPA (LOD= 0.001 mg/kg). The results confirmed that DPA was present in its biomass sample (0.0027mg/kg) and oleoresin samples (0.1-0.3 mg/kg) at levels close to the limit of detection of the screening method.

23. On further investigation, the applicant identified the rubber pipes of two peristaltic pumps which serve the downstream processing unit and the roll of the diaphragm pump of the culture room as the source of DPA contamination. The applicant resolved this issue by replacing the contaminating equipment with DPA free versions. The applicant also highlighted that for an individual to ingest more than the ADI for DPA of 0.08 mg/kg bw (equivalent to 4.8 mg/day for a 60kg adult), they would have to consume more than 24 kg of oleoresin daily.

Microbiological contamination

24. The applicant routinely tests batches of algal meal and oleoresin for microbiological contamination using methods compliant with German national standards (64 LFGB German Food Act, DIN). Analyses include Total Plate Count, detection of yeasts and moulds, coliforms, *E. coli*, *S. aureus*, *Pseudomonas sp*. and *Salmonella*. The applicant has provided a test report and certificates of analysis for 4 batches of product in Appendix 8.

Discussion: *The Committee was satisfied that the applicant had quality control procedures in place to minimise the risk of contamination of the algal culture and the oleoresin and noted the absence of trace elements, pesticides and microbiological contamination as a result of their analysis. The Committee also noted that, in addition to its approved uses as a pesticide on certain fruits, DPA is widely used as an antioxidant in the lubricant, rubber and plastics additive industries and it can therefore enter the food chain via number of routes. The Committee was content that tests carried out on the cultivating system following the replacement of the contaminating equipment confirmed that the DPA issue had been resolved by the manufacturer.*

Additional information

25. **Manufacturing quality control:** The applicant's facilities are certified as compliant with State of Israel Department of Health Good Manufacturing Practices standards and are permitted to use the official GMP symbol on their products. The algal meal and NI are produced in certified ISO 9001

production facilities and externally audited HACCP quality control management systems are in force. The applicant has provided certificates of compliance with GMP, ISO and HACCP principles.

26. The analytical laboratory used by the applicant in its quality control programmes, Bactochem Ltd., is ISO 9001 accredited by the Israel Laboratory Accreditation Authority for all chemical and microbiological assays cited.

27. The applicant also states that its products are free from genetically modified organisms, free from sources of bovine spongiform encephalopathy and have not been treated with ionising radiation.

28. **Labelling:** The applicant has provided a sample copy of their product label.

29. **Stability:** In light of a comment received during the public consultation on this dossier, additional information was provided to show that the carotenoids in the oleoresin are stable at two temperatures. These results indicated that the carotenoid content of the NI is stable for 12 months when stored at 5°C and is subject to losses of less than 10% when stored at 25°C. The applicant states that the maximum acceptable losses over the shelf life of carotenoid products is considered to be 10%. Extrapolation of the 5° trend line to 18 months indicates that the retention is greater than 95%. The applicant intends to advise purchasers of the NI to keep the product in tightly closed containers in a cold, dry, dark location; preferably below 5°C. The applicant states that under these conditions a shelf life in excess of 18 months is anticipated. The applicant also notes that cost-effective stock management means that users are unlikely to store such products for extended periods of time.

30. The applicant highlights that the NI is made from the same starting material and extracted using the same technology as Valensa's oleoresin. The applicant is of the view that the two products are chemically indistinguishable and that their NI can therefore be assumed to be nutritionally and metabolically equivalent to Valensa's extract and also to the whole algal meal.

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

CONCLUSION

31. The Committee noted that the applicant's product is manufactured from exactly the same starting material as the existing product and that the extraction methods used by the two manufacturers were very similar. It was therefore not anticipated that the end products would be substantially different and this was confirmed by analysis of the composition of a limited number of batches of the two products. In other circumstances the Committee would expect claims of substantial equivalence to be based on a larger number of samples, in order to allow formal statistical analysis of the

results. However, in this case the low number of replicates was offset by the use of the identical starting material and the similarities between the manufacturing methods.

32. The Committee therefore concluded that Algatechnologies (1998) has demonstrated the equivalence of their astaxanthin-rich oleoresin obtained from *H. pluvialis* with the existing astaxanthin-rich *H. Pluvialis* oleoresin according to the criteria set out in Article 3(4) of the Novel Foods Regulation (EC) 258/97.

33. This opinion applies solely to the specified product, produced according to the processes described in the dossier, to be used by food supplement manufacturers with an astaxanthin content of no more than 4mg per capsule or tablet.

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