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Substantial Equivalence Application for the Approval of an Astaxanthin rich Carotenoid Oleoresin, Parry's AstaNatural derived from algae Haematococcus pluvialis for use in Human Dietary Supplements

Application submitted by Parry Nutraceuticals, Division of EID Parry (India) Ltd, India.

Introduction

Parry Nutraceuticals produces Haematococcus pluvialis algae cell powder for the past few years. The cracked cell powder has been exported to many countries including EU. Now, Parry Nutraceuticals intends to produce Astaxanthin Oleoresin, named as AstaNatural, from the cracked cell powder and market in EU for use in dietary supplements. The oleoresin will be available in three different concentrations (2.5, 5.0 and 7.0%). Parry also intends to market in the form of bulk softgel with astaxanthin level not more than 4mg/softgel. Two different brands
of astaxanthin oleoresin were available in EU market. One produced by Valensa Inc (formerly US Nutra) with a brand name Zanthin and the other by Cyanotech Corporation, with a brand name BioAstin. BioAstin is available in two different concentrations, BioAstin SCE5 and BioAstin SCE10 (5 and 10%).

Approval of Parry's AstaNatural is sought under Article 5 of EC regulation No. 258/97, which deals with the introduction of novel foods and ingredients into the EU and ensures that the novel food in question is assessed for its safety prior to introduction. The Application and data or documents were prepared as per the "ACNFP guidelines for the presentation of data to demonstrate substantial equivalence between a novel food or food ingredient and an existing counterpart". Equivalence is made with Cyanotech's BioAstin. Information and data were presented in this application to demonstrate that Parry's AstaNatural is similar to Cyanotech's BioAstin.

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1. Administrative Information

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1.3. Name of Novel Food Ingredient

Parry's **AstaNatural**, an oleoresin of Haematococcus pluvialis microalgae

1.4. Date of Application

2. Composition

2.1 Chemistry, sources, distribution of astaxanthin

Astaxanthin (3, 3'–dihydroxy–b, b–carotene-4, 4'-dione) is widely distributed in nature and is the principle pigment in crustaceans and Salmonoids, various birds including flamingoes and scarlet ibis. The carotenoids impart distinctive orange – red colouration (Bjerkeng, 1997). Astaxanthin is a keto (oxygenated) carotenoids with the molecular formula $C_{40}H_{52}O_4$ and has a molecular weight of 596.86.

Astaxanthin exists in several stereochemical forms viz 3S, 3'S; 3R, 3'R; 3S, 3'R and 3R, 3'S depending on the source (Schiedt et al., 1981). The major natural sources of astaxanthin include algae Haematococcus, yeast Phaffia and Krill apart from salmon and trout. Among these, Haematococcus was the richest sources producing between 1 to 4% of its dry weight (Maher, 2000). In the algae astaxanthin exists as 3S, 3'S isomer whereas in the yeast it is in the form of 3R, 3'R isomer.

Astaxanthin exists in Haematococcus cells in the form of monoester and diester, which accounts for more then 90% of total astaxanthin. Other carotenoids such as beta-carotene, canthaxanthin and lutein were present in minor amounts (Lee and Zhang, 1999). In synthetic astaxanthin and in Phaffia it is in free form.

2.2 Specifications

The general composition Parry's AstaNatural oleoresin is as follows:

TABLE: 1
General composition and specification of AstaNatural

Parameter	AstaNatural 2.5%	AstaNatural 5.0%	AstaNatural 7.0%
Protein	<3%	<3%	<3%
Carbohydrate	<2%	<2%	<2%
Lipid	90-95%	90-95%	90-95%
Ash	2-4%	2-4%	2-4%
Moisture	<5%	<5%	<5%
Total Astaxanthin	>2.5%	>5.0%	>7.0%

2.3 Classification and strain of Haematococcus

The algae Haematococcus pluvialis, which is used for the preparation of Parry's AstaNatural oleoresin, is a freshwater one and the taxonomic classification is as follows:

Class:	Chlorophyta
Order:	Volvacales
Family:	Volvacacea
Genus:	Haematococcus
Species:	pluvialis

Under optimal growth conditions the cells are green and ellipsoidal, the two flagellates provide motility and cells divide rapidly. The synthesis of red pigment astaxanthin occurs during stress (chemical and environmental) during which time the cells become non- motile (aplanospore). The formation of resting cells and the accumulation of astaxanthin have been considered to protect the assimilatory pigment, chlorophyll from damage due to high light intensities.

There are various species and strains of Haematococcus available in leading culture collection centres and Haematococcus pluvialis is the common species used by most commercial organizations (Cohen et al., 2000). The strain and species used by Parry is obtained from SAG culture collection centre, Gottingen. The species is Haematococcus pluvialis flotow (CONFIDENTIAL). The mother culture is maintained in its pure form at Parry's Research and Development Unit for several years and no genetic modification has been done at any time. Astaxanthin products available in European market such as Zanthin, BioAstin and AstaReal were obtained from Haematococcus pluvialis (same species but strain may be different) cracked cells similar to Parry's. There are no major changes in the profile of astaxanthin between different strains.

2.4 Cultivation of Haematococcus pluvialis algae

Parry's production facility is located in a remote hamlet in Southern India. The facility is bestowed with uncontaminated fresh water and there is no agricultural activity around the facility. Thus there is no contamination from ground, water or air.

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The facility is equipped with water treatment plant, separate raw material store, well maintained harvesting and drying systems and a full fledged QA lab to take care of all the regular analysis needed for analyzing the product and monitor the cultivation.

A flow chart and description on the cultivation, harvesting and drying of Haematococcus cell powder is enclosed separately (CONFIDENTIAL- Appendix -1).

Parry's production mode is open raceways type. The algae is grown in open raceway ponds containing medium with required amount of nutrient for growth. Astaxanthin production was induced by nutrient limitation and sunlight. On completion of astaxanthin synthesis, the cells were harvested by filtration technique; adhered chemicals removed by washing, cell biomass re-concentrated and then spray dried. Cultivation by producers like Algatech and Astacarotene (Sweden) are carried out in closed photobioreactors. Cyanotech cultivation method involves two stages, growth stage in indoor closed photo bioreactors and astaxanthin induction stage in open raceway ponds similar to ours.

The following parameters are monitored to ensure that the culture is monoalgal and the extracted material is free of contaminants: optimum light intensity by shading, culture depth, limitation of nutrient, inoculum size and selective harvesting to capture only Haematococcus cells.

Well established HACCP system, ISO 9001 and ISO 14000 system in place to take care of food safety, quality and environmental aspects. Other players in this field such as Fuji from Japan and Algatech from Israel uses closed system. Apart from production mode, there is no major difference in astaxanthin profile in cells grown under different conditions.

2.5 Extraction method

Parry's AstaNatural oleoresin is extracted from cracked Haematococcus algae cell powder.

The process flow chart for preparation of Astaxanthin oleoresin is attached in Appendix -2 (CONFIDENTIAL). The cells were cracked using a proprietary process ensuring about 90% cracking without subjecting the cells to oxidation. Astaxanthin from the cracked cells were extracted using food grade solvent ethyl acetate. The extraction process is carried out under a nitrogen atmosphere. Extracted astaxanthin is separated from the spent powder by centrifugation. Special care is taken to remove fine cell debris from the extract. The extract is then subjected to a three stage distillation process under different vacuum conditions to ensure that the final oleoresin will have solvent residue below the specified limit. Other major producers of oleoresin use supercritical extraction method (Zanthin and BioAstin SCE). Use of solvent and the residue in the final oleoresin is being monitored for every batch and the limit has been fixed as below 100ppm. The results were also counterchecked regularly at accredited labs to make sure the specification is complied all the time. Dilution of oleoresin if required to 2.5%, 5.0% were made with high oleic olive oil and then packed under nitrogen atmosphere in food grade containers.

2.6 Composition of the final product

Parry plans to make three different concentrations of AstaNatural oleoresin viz 2.5%, 5.0% and 7.0% according to the market demand and specific customer request. Each batch of these products will be subjected to regular analysis both chemical and microbiological. Moreover, by monitoring the culture conditions and extraction parameters batch to batch consistency is being maintained both for cell powder and astaxanthin oleoresin. The following table gives the general composition of Astaxanthin oleoresin (average of three lots).

TABLE: 2: General Composition of AstaNatural

Parameter	AstaNatural 2.5%	AstaNatural 5.0%	AstaNatural 7.0
Protein	2.7±0.20	0.95±0.03	0.91±0.02
Carbohydrates	0.32±0.02	0.11±0.01	0.10±0.01
Lipid	93.93±0.23	94.89±0.12	94.95±0.18
Ash	2.77±0.02	3.82±0.08	3.79±0.19
Moisture	0.2±0.03	0.23±0.02	0.21±0.04
Total Astaxanthin	2.54±0.03	5.14±0.04	7.22±0.10
Solvent residue, ppm	BDL	2.26±0.67	6.38±2.09

(Appendix-3A. General composition (GC) of AstaNatural.
Appendix-4. Total Astaxanthin percentage in AstaNatural).

Astaxanthin is analysed by spectrophotometry and further confirmed by HPLC. The methods used by Parry are similar to Cyanotech (BioAstin) and the results are also compared with another producer of astaxanthin Algatech. All the methods give similar values. By spectrophotometric method total carotenoids present in the algae powder or Astaxanthin Oleoresin is calculated. The percentage of total astaxanthin (all-trans and cis isiomers) will be not less than 90% of total carotenoids, as confirmed by HPLC method.
The HPLC method was first published in Journal of High Resolution chromatography (Vecchi et al., 1987). Later on it was adopted and validated by Hoffmann La Roche for synthetic astaxanthin. The method is published in the Roche Bulletin "Determination of Stabilised Astaxanthin in Carophyll Pink, Premixes and Fish Feeds" in 1995. The method is also been adopted by Canadian Food Inspection Agency analytical laboratories for registration of Haematococcus algal meal in salmonid feeds (Registration Number 990535) and later by US Food and Drug Administration (21 CFR 73.185).

Producers of this alga worldwide use this method for calculating astaxanthin content. Since there is no validated method to estimate astaxanthin esters, the oleoresin is first treated with an enzyme to cleave the ester and preceded for HPLC.

Astaxanthin in Parry's Haematococcus cell powder and oleoresin presents mainly in the form of esters similar to other producers. This is specific to Haematococcus species and literature shows that there is no major difference between strains. Also it is noticed that the type of cultivation or extraction process does not influence the ester composition significantly. It is present in the form of monoester (about 70%), diester (about 10%) and less than 5% in the free form. Apart from these, the algae also produce minor amounts of betacarotene, lutein and canthaxanthin. Parry's cultivation process ensures that the astaxanthin biosynthesis is complete before harvesting so that major carotenoid will be astaxanthin.

(Appendix-5. Determination of Total astaxanthin in AstaNatural by spectroscopic method.
Appendix-6. Determination of Astaxanthin by HPLC method)

Fatty acid analysis was done in-house by Gas chromatography. Methyl esters of fatty acids were expressed on area percent basis.

Solvent residue analysis in Astaxanthin Oleoresin was carried out by in-house method using GC-Headspace chromatography with an auto injector. Quantification was done using pure solvents by creating a standard curve. Residue analysis was carried out for all the batches to check the limits and every month samples are being sent to accredited labs for counterchecking. Parry's HACCP system takes solvent residue in Astaxanthin Oleoresin as a Critical Control Point and monitoring is done on a day-to-day basis to ensure compliance and to make further improvement.

2.7. Comparison of Astaxanthin Oleoresin with Haematococcus cell powder

Analytical results of astaxanthin Oleoresin were compared with that of the cell powder from which it has been extracted. This is to show that the oleoresin contains the same astaxanthin profile as in the powder and the overall quality of the astaxanthin is similar to cell powder. The table below will show the comparison of astaxanthin profile (average of three lots).

TABLE: 3
Comparison of Astaxanthin profile (area percent)

Carotenoids	Cell powder	AstaNatural 2.5%	AstaNatural 5.0%	AstaNatural 7.0%
Betacarotene	0.62±0.01	0.62±0.01	0.62±0.01	0.61±0.01
Canthaxanthin	1.21±0.03	1.21±0.02	1.20±0.03	1.10±0.17
Astacene	3.09±0.06	3.09±0.06	3.09±0.06	3.06±0.06
Semiastacene	1.35±0.03	1.32±0.03	1.35±0.03	1.34±0.03
Dicis astaxanthin	1.07±0.02	1.02±0.02	1.03±0.05	1.06±0.03
Trans astaxanthin	75.70±1.53	75.78±1.53	75.75±1.51	74.87±1.48
9 cis astaxanthin	9.20±0.77	9.19±0.77	9.19±0.77	9.65±0.78
13 cis astaxanthin	6.10±0.94	6.08±0.94	6.08±0.93	6.63±0.94
Lutein	1.66±0.03	1.62±0.03	1.65±0.03	1.64±0.03

(Appendix-7. Astaxanthin profile of Haematococcus cell powder and AstaNatural oleoresins)

Fatty acid profiles of different concentration of AstaNatural oleoresin were also compared with dry algal powder. This is just to show that all the fatty acids present in Haematococcus algal powder were present in oleoresin. There will be some variation in the content of fatty acid because of dilution of higher concentration oleoresin with high oleic olive oil. This variation will not have any significant effect on the nutritional properties of the oleoresin.

TABLE: 4
Comparison of Fatty acid profile

Fatty acid	Cell Powder	AstaNatural 2.5%	AstaNatural 5.0%	AstaNatural 7.0%
14:0	0.37±0.03	0.24±0.04	0.23±0.05	0.32±0.07
15:0	------	0.01±0.01	0.1±0.06	0.1±0.06
16:0	26.93±0.85	20.45±0.33	24.57±0.84	25.59±0.95
16:1	0.21±0.15	-----	0.57±0.20	0.54±.021
16:2	0.66±0.02	0.23±0.33	0.45±0.12	0.58±0.08
16:3	0.25±0.01	0.18±0.30	0.14±0.06	0.20±0.05
16:4	1.60±0.08	-----	1.15±0.10	1.47±0.01
17:0	3.02±0.19	2.91±0.07	2.14±0.15	2.72±0.24
18:0	1.98±0.10	1.41±0.16	1.61±0.21	1.80±0.26
18:1	35.77±0.49	53.53±0.12	38.93±1.65	35.65±2.36
18:2	17.03±0.39	12.11±0.14	17.22±0.45	16.56±0.51
18:3, n 6	0.67±0.58	-----	0.84±0.30	0.95±0.15
18:3, n 3	8.98±0.37	6.49±0.05	8.14±0.30	8.38±0.37
18:4	0.74±0.68	0.74±0.03	1.30±0.50	0.81±0.71
20:2	0.78±0.42	0.66±0.05	0.81±0.20	0.88±0.10
20:4	0.40±0.27	0.05±0.09	0.85±0.32	0.68±0.26
22:0	1.31±0.53	0.11±0.19	0.50±0.20	1.04±0.72

(Appendix-8. Fatty acid profile of Haematococcus cell powder and AstaNatural oleresins)

2.8 Substantial equivalence of Parry's AstaNatural with an equivalent product

Parry intends to compare its AstaNatural Oleoresin products with that of BioAstin produced by Cyanotech Corporation for which equivalence was granted in Feb 2007. Cyanotech produces Astaxanthin Oleoresin in two formulations, 10% and 5% named as BioAstin SCE10 and BioAstin SCE5 (i.e. 10% and 5% astaxanthin products). Parry intends to produce three formulations 7%, 5% and 2.5% Astaxanthin Oleoresin. High oleic olive oil is used as diluent for lower concentration products. Another producer of astaxanthin oleoresin is Velensa Inc, who produces 10% astaxanthin oleoresin.

A comparison of proximate, fatty acid profile and astaxanthin profile has been made to show the similarities between Parry's and Cyanotech's astaxanthin, both in powder and oleoresin form (Cyanotech's data obtained from the substantial equivalence application filed by them which is available on the internet). From the table it is clear that the both Parry and Cyanotech powder contains all the constituents. The level of individual constituents vary because of the culture conditions. As mentioned earlier, the growth stage by Cyanotech is carried out in controlled indoor conditions, whereas, Parry does this operation in outdoor open ponds . May be because of this there is some variation in the major cell constituents. This difference will not have any major impact in the extraction of oleoresin. From the table below it is clear that the astaxanthin content in both the powder were lower than others. (Appendix -10, comparison of Parry's cell powder with Cyanotech's – analysed in same lab). This is mainly because Parry's entire cultivation is carried out in open ponds and Cyanotech uses open ponds only for pigment induction. Since we don't have much control in open environment during growth stage and astaxanthin accumulation stage, the pigment levels are lower. Hence, it is the method of cultivation and not the strain of the algae which determines the astaxanthin content in the final product. Parry opted for open cultivation since it has earlier experience with different algae and also to reduce the cost of production and investment. Hence, Parry's and Cyanotech's powder are similar in terms of proximate composition.

TABLE: 5

Comparison of proximate composition – Haematococcus cell powder

Parameter	Parry (%)	Cyanotech (%)
Protein	15.5±1.4	28.6±1.3
Carbohydrates	47.0±3.5	44.8±3.3
Lipid	20.0±2.1	13.1±0.1
Fiber	27.4±2.5	19.2±1.1
Ash	14.4±1.6	6.70±1.1
Moisture	3.0±1.5	6.80±1.0
Total Astaxanthin	1.52±0.1	2.58±0.1

Appendix 9A – 9D: Specification sheet

a. Appendix-9A: Parry's Haematococcus cell powder
b. Appendix-9B: Parry's AstaNatural 2.5% -
c. Appendix-9C: Parry's AstaNatural 5%
d. Appendix-9D: Parry's AstaNatural 7%

Appendix-10. General composition of Parry's Haematococcus cell powder – average of three lots and comparison chart of recent sample)

TABLE: 6
Comparison of fatty acid profile

Fatty acid	Cell Powder Parry	Cell powder Cyanotech
14:0	0.37±0.03	0.6±0.02
15:0	------	0.2±0.02
16:0	26.93±0.85	24.70±0.16
16:1	0.21±0.15	1.0±0.13
16:2	0.66±0.02	NA
16:3	0.25±0.01	NA
16:4	1.60±0.08	NA
17:0	3.02±0.19	0.2±0.0
18:0	1.98±0.10	1.2±0.13
18:1	35.77±0.49	27.5±0.16
18:2	17.03±0.39	29.5±1.08
18:3, n 6	0.67±0.58	2.3±0.12
18:3, n 3	8.98±0.37	NA
18:4	0.74±0.68	NA
20:2	0.78±0.42	0.5±0.03
20:4	0.40±0.27	1.1±0.03
22:0	1.31±0.53	0.3±0.01

Table 6 shows a comparison of fatty acid profile in parry powder and cyanotech powder. From the table, it is clear that the major fatty acids are same for both Parry powder and Cyanotech powder. As said earlier, the differences in the values may be due to the mode of cultivation and the medium used. We feel that Cyanotech powder also has 18:3, n-3 as a major fatty acid similar to Parry's. This is confirmed from their undiluted oleoresin (BioAstin SCE 10) which was shown to have the above fatty acid as a major constituent. Thus, the fatty acid profile is comparable to Cyanotech showing that both the powder will have similar lipid molecules.

TABLE: 7

Comparison of carotenoid profile:

Carotenoids	Cell powder Parry	Cell powder Cyanotech
	Percent of total weight	
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
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

Table 7 shows the comparison of carotenoids profile in parry powder and cyanotech powder. From the table it is clear that trans, 9 cis and 13 cis astaxanthin were the major carotenoids in both the powder. The slight difference in individual astaxanthin value and that of astaxanthin precursors such as lutein, betacarotene and canthaxanthin are due to change in the biosynthesis (mainly based on harvest time). If the astaxanthin biosynthesis is not complete its precursors such as lutein and betacarotene content will be more.

From the above data, it is clear that the chemical composition of parry cell powder is comparable to cyanotech cell powder.

In addition, comparison was made with parry's AstaNatural with that of BioAstin with respect to proximate composition, fatty acid profile and carotenoid profile. The proximate composition of oleoresin is different in the sense that Parry's AstaNatural will have more lipid content. The fatty acid profile in the oleoresin is similar to the powder in the undiluted oleoresin (Appendix - 8).

TABLE: 8
Comparison of Proximate composition – Astaxanthin Oleoresin

Parameter	AstaNatural 7.0%	BioAstin 10.0%
Protein, %	0.91±0.02	3.3±1.1
Carbohydrates, %	0.10±0.01	47.3±5.5
Lipid, %	94.95±0.18	42.8±0.6
Ash, %	3.79±0.19	1.2±1.4
Moisture, %	0.21±0.04	5.5±2.5
Total Astaxanthin, %	7.22±0.10	10.57±0.43

(Refer Appendix-3Aand Appendix 4)

This difference is mainly due to type of extraction used by Parry. Since Parry uses a solvent extraction method, the lipid content is high. BioAstin is produced via supercritical extraction (and hence the level of carbohydrate is high). But this difference does not have any effect on the bioavailability or functioning of astaxanthin. Even with same extraction method, there will be some changes. For example, even though US Nutra carries out their process by supercritical extraction, we notice from your advisory committee report that their extract contains about 89.2% fatty acids and 10.2% carotenoids. This composition is similar to our AstaNatural. Parry assures that other than concentrating lipid, using solvent extraction, no other unwanted contaminants will be selectively concentrated.

The fatty acid profile of BioAstin and Parry's AstaNatural are similar, since there is no significant difference in the fatty acid profile between different strains of Haematococcus or culture methods. Slight variation in individual fatty acids will be inevitable because of the culture conditions. Moreover, Parry is not sure if BioAstin 10% is a diluted one or undiluted one. If it has been diluted there will be some variation because of the diluent oil.

The HPLC profile of astaxanthin in Parry's Astaxanthin is very similar to Cyanotech's BioAstin. Astaxanthin is above 90% of total carotenoids and the levels of astaxanthin degradation products, astacene and semiastacene are kept below 5%. The total astaxanthin level may vary from 80 to 90% depending on the completion of biosynthesis in the algae before harvest. As said earlier, there is no difference in the distribution of astaxanthin esters. The presence of smaller amounts of other carotenes such as betacarotene, canthaxanthin and lutein were noticed in both the products.

TABLE: 9 :
Fatty acid profile – Comparison of Astaxanthin Oleoresins (area percent).

Fatty acid	AstaNatural 2.5%	AstaNatural 7.0%	BioAstin 5.0%	BioAstin 10%
14:0	0.24±0.04	0.32±0.07	0.3±0.04	0.4±0.07
15:0	0.01±0.01	0.1±0.06	0.2±0.11	BDL
16:0	20.45±0.33	25.59±0.95	12.0±0.97	19.6±0.38
16:1	NA	0.54±0.02	0.5±0.09	1.0±0.16
16:2	0.23±0.33	0.58±0.08	NA	NA
16:3	0.18±0.30	0.20±0.05	NA	NA
16:4	NA	1.47±0.01	NA	NA
17:0	2.91±0.07	2.72±0.24	0.1±0.04	0.2±0.00
18:0	1.41±0.16	1.80±0.26	1.5±0.43	1.9±1.02
18:1	53.53±0.12	35.65±2.36	44.1±16.51	24.9±2.30
18:2	12.11±0.14	16.56±0.51	20.0±0.17	32.8±4.60
18:3, n 6	NA	0.95±0.15	0.9±0.25	1.4±0.59
18:3, n 3	6.49±0.05	8.38±0.37	12.1±3.63	14.3±1.79
18:4	0.74±0.03	0.81±0.71	NA	NA
20:2	0.66±0.05	0.88±0.10	0.2±0.11	0.3±0.00
20:4	005±0.09	0.68±0.26	0.5±0.18	0.6±0.13
22:0	0.11±0.19	1.04±0.72	0.3±0.03	0.2±0.00

(Refer Appendix-8)
BDL – Below detection limit
NA – Not available

TABLE: 10 :
Astaxanthin profile – Comparison of Astaxanthin Oleoresin (percent of total weight)

Carotenoids	AstaNatural 5.0%	AstaNatural 7.0%	BioAstin 5.0%	BioAstin 10%
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

(Refer Appendix-7)

The above data shows that Parry's AstaNatural oleoresin is comparable to BioAstin produced by Cyanotech.

3. Nutritional value:

3.1 General description on carotenoids

They are different types of carotenoids found in nature. They are widely distributed in vegetables and fruits, algae and fungi. In nature it occurs mainly as carotenes and xanthophylls. The biological functions of carotenoids includes source of vitamin A, protection against oxidation of essential fatty acids, protection against UV light, immune response etc., (Guerin et al., 2003). The major carotenoids whose benefit has reached the market include Betacarotene, Lutein, Zeaxanthin, Lycopene, Astaxanthin and more recently Fucoxanthin.

3.2 History of use of astaxanthin

Astaxanthin is one of the xanthophyll groups of the carotenoids. It is closely related to Xanthophylls zeaxanthin and lutein and hence shares many of the metabolic and physiological functions attributed to them. The presence of hydroxyl and keto endings on each ionone ring, explains some of the unique features, such as the ability to be esterified, high antioxidant potential and polarity configuration (more polar) than other carotenoids. Earlier use of astaxanthin in human diet is mainly through marine sources such as salmon, lobster, rainbow trout, shrimp, crawfish, crab, and red caviar all of whom owe their rich colors to the presence of astaxanthin. But the level of astaxanthin is in these sources are in ppm levels.

Astaxanthin from microalgal cell powder (from Haematococcus species) has been in the market for quite some time and particularly in Europe prior to 1995 as dietary supplement. Then the oleoresin version and related formulations entered world market in late 1990's and early 2000, mainly in US and then in EU. Currently Astaxanthin oleoresin is available from Fuji Chemical Industry (AstaReal), US Nutra, now Valensa Inc (Zanthin), Cyanotech Corporation (BioAstin) and Algatech (Astapure). Other commercial parties include Micro Gaia Inc, Aquasearch (from Haematococcus), Neptune Technologies and Bioresources (from Krill) and Igene Biotechnology (from yeast Phaffia rhodozyma).

Thus the use of Haematococcus cell powder or its oleoresin as human supplement is not new.

4. Metabolism

4.1 Bioavailability

Carotenoids are absorbed in the intestine with dietary fat and are stored primarily in the lipid portions of the body. Approximately 80 percent of carotenoids are distributed in adipose tissues, with smaller amounts found in the liver, muscle, adrenal glands and reproductive organs.

In the plasma, beta- and alpha-carotene or lycopene, are mostly transported by very low-density lipoproteins (VLDLs) and low-density lipoproteins (LDLs), while astaxanthin, zeaxanthin, or lutein, are more likely to be transported by LDLs and high-density lipoproteins (Deming and Erdman, 1999).

The process of nutrient absorption requires movement of the digested food components into the mucosal cells of the intestinal wall. Uptake occurs when the xanthophyll or its metabolites enter the intestinal mucosal cells. Absorption is achieved with the movement of the xanthophyll or its bioactive metabolite through the mucosal cells into the portal or lymphatic system. Xanthophyll bioavailability can be defined as the proportion of the ingested xanthophyll that is made available (i.e., delivered to the bloodstream) for its intended mode of action.

The stages involved in the optimal absorption of xanthophylls are: 1. Release of xanthophylls from the food matrix. 2. Transfer of xanthophylls to lipid micelles in the small intestine 3. Uptake of xanthophylls by intestinal mucosal cells and 4. Transport of xanthophylls or their metabolic products to the lymph system.

Scientific studies have revealed that Astaxanthin is well absorbed even at a dosage of 100mg and its transport in the plasma by lipoproteins. It is important to note that dietary fats and oils are crucial along with other factors like protein, fibre etc., for the regular body's metabolism like better absorption. This dietary fat will ensure proper absorption of carotenoids. This fact has been ascertained by a study, which revealed that Astaxanthin absorption was enhanced when given in lipid base formulation (Osterlie et al., 2000). A review on human bioavailability studies on xanthophylls can be obtained from Susan and Erdman (2002).

Given below are few studies on the bioavailability of Astaxanthin.

1. Effect of route of administration and carrier on bioavailability and kinetics of astaxanthin in Atlantic salmon Salmo salar L (Maltby et al., 2003).
   This study examined astaxanthin bioavailability and kinetics in adult Atlantic salmon Salmo salar L., following two different routes of astaxanthin administration (oral vs. intraperitoneal (i.p.) injection) using two different carriers of the pigment (gelatin vs. sesame oil). The dorsal aorta of adult Atlantic salmon (mean initial weight 950 g) was cannulated. The fish received a single dose of astaxanthin (572 µg kg1) in sesame oil or (514 µg kg1) in gelatin via the oral or i.p. route. Plasma was sampled regularly up to 72 h post oral administration and up to 510 h post i.p. injection. The astaxanthin concentration–time curves from plasma were best fit to a one-compartment pharmacokinetic model for each of the four treatments. The gelatin carrier resulted in higher availability of astaxanthin compared to the sesame oil carrier. The bioavailability for astaxanthin in sesame oil was only 38.7% of that in gelatin by i.p. injection, and only 53.5% of that in gelatin by oral administration. Higher availability of astaxanthin was observed when i.p. injection was used compared to oral administration. The bioavailability for astaxanthin administered orally was only 12% of that by i.p. injection in sesame oil, and only 8.7% of that by i.p. injection in gelatin.

2. Oral bioavailability of the antioxidant astaxanthin in humans is enhanced by incorporation of lipid based formulations (Odeberg et al., 2003).
   In this open parallel study, healthy male volunteers received a single dose of 40 mg astaxanthin, as lipid based formulations or as a commercially available food supplement, followed by blood sampling for further analysis of plasma concentrations. Pharmacokinetic parameters were calculated to evaluate the extent and rate of absorption from each formulation. The elimination half-life was 15.9±5.3 h (n=32), and showed a mono-phasic curve. Three lipid based formulations: long-chain triglyceride (palm oil) and polysorbate 80 (formulation A), glycerol mono- and dioleate and polysorbate 80 (formulation B), and glycerol mono- and dioleate, polysorbate 80 and sorbitan monooleate (formulation C), all showed enhanced bioavailability, ranging from 1.7 to 3.7 times that of the reference formulation. The highest bioavailability was observed with formulation B, containing a high content of the hydrophilic synthetic surfactant polysorbate 80.

3. Effect of esterification on the absorption of astaxanthin in rainbow trout, Oncorhynchus mykiss (Walbaum) (White et al., 2002).
   Gastrointestinal and serum absorption of astaxanthin was studied in rainbow trout, Oncorhynchus mykiss (Walbaum) (217 ± 2 g) fed diets supplemented with either esterified astaxanthin (from Haematococcus pluvialis) or free astaxanthin (synthetic, as 8% w/w beadlets) at similar levels (50 mg kg-1). After 56 days of feeding, there was a significant difference (P = 0.0582) between steady-state serum astaxanthin concentrations for fish fed free (2.0 ± 0.3 g mL-1) or esterified astaxanthin (1.3 ± 0.1 g mL-1) at the 90% confidence level. However, following ingestion of a single meal supplemented with free or esterified astaxanthin, the rates of astaxanthin absorption into serum were not significantly different (P > 0.1) (0.8 ± 0.2 µg mL-1 h-1 and 1.0 ± 0.4 µg mL-1 h-1 respectively). In fish fed both free or esterified astaxanthin, higher absorption (P < 0.05) of astaxanthin by the ileal (0.8 ± 0.14 g g-1 and 0.9 ± 0.15 g g-1 respectively) compared with the posterior (0.2 ± 0.01 g g-1 and 0.3 ± 0.14 g g-1 respectively) intestine was recorded. This confirmed the role of the anterior intestine in carotenoid absorption. Non-detectable levels of esters in digesta taken from the hind intestine suggest the anterior intestine is also the primary region for ester hydrolysis.

4. Plasma appearance and distribution of astaxanthin E/Z and R/S isomers in plasma lipoproteins of men after single dose administration of astaxanthin (Osterlie et al., 2000).
   Appearance, pharmacokinetics, and distribution of astaxanthin E/Z and R/S isomers in plasma and lipoprotein fractions were studied in 3 middle-aged male volunteers (37-43 years) after ingestion of a single meal containing a 100 mg dose of astaxanthin. The results indicate that a selective process increases the relative proportion of astaxanthin Z-isomers compared to the all-E-astaxanthin during blood uptake and that astaxanthin E/Z isomers have similar pharmacokinetics.

5. Bioavailability of all-E-astaxanthin and Z-isomers of astaxanthin in rainbow trout (Oncorhynchus mykiss) (Bjerkenga et al., 1997).
   Two cold-pelleted diets containing either 36.9 or 35.4 mg kg-1 of predominantly all-E-astaxanthin (97% of total astaxanthin) or a mixture of all-E- and Z-astaxanthin (64 and 36%, respectively), were fed to duplicate groups of rainbow trout (Oncorhynchus mykiss) in freshwater (initial weight 0.4 kg) for 69 days. The results indicate that the Z-astaxanthin isomers are not utilized to the same extent as all-E-astaxanthin for flesh pigmentation. No significant differences were observed in total astaxanthin retention in the flesh. However, the retention of all-E-astaxanthin was higher in trout fed the stereoisomer mixture of astaxanthin than in the group fed all-E-astaxanthin. The results also indicate selectivity in metabolism of the different stereoisomer of astaxanthin, either during absorption, transport or deposition, or their combinations and suggest that substantial isomerization of astaxanthin Z-isomers occurs in the liver.

4.3 Stability of the product

Parry's strict monitoring of the process ensures that the oleoresin is stable for a minimum period of one year (Appendix-11. Stability data - AstaNatural). Parry takes great care throughout the extraction process which includes selection of powder, mode of cracking and extraction and concentration. All the processes were done under nitrogen atmosphere and under vacuum. Commercially available products also indicate a stability of one year.

5. Intended use

As mentioned earlier astaxanthin is initially consumed through Salmon. From the studies on daily consumption, it was found that a level ranging from 2-12mg was consumed worldwide without any adverse effect (John West Food Products, 2002). The level in the dried algal meal sold in the market is in the range of 2-5mg.

Parry plans to market its astaxanthin oleoresin to be used in soft gelatin and formulations by human dietary supplements market. As mentioned earlier Parry intend to market its astaxanthin oleoresin in three different formulations viz 2.5, 5.0 and 7.0%. As per the EU regulation the level will not be more than 4 mg per softgel. Parry also intends to market bulk softgels depending on the market demand.

Similar products have been approved in Europe for use in dietary supplements. They include Zanthin, AstaReal and BioAstin. Parry's intended level of use is equivalent to these products.

6. Level of undesirable substances

6.1 Introduction

Parry's regular monitoring of the cultivation conditions, screening of nutrients and process ensures that the level of undesirable substances are well below the specified limits (BioAstin and Zanthin values obtained from Cyanotech's and US Nutra's substantial equivalence application).

6.2 Heavy metals

The level of heavy metals in the three different formulations is below the specified limits. The table below shows that it is equivalent to the existing products like Zanthin and BioAstin.

TABLE: 11
Comparison of heavy metals

Heavy metal	AstaNatural	BioAstin	Zanthin
Lead	<0.5ppm	0.30ppm	<0.5ppm
Arsenic	<0.5	1.19	<0.5
Cadmium	<0.5	<0.1	<0.5
Mercury	<0.025	<0.025	<0.025

(Appendix -12. Heavy metal analysis report of AstaNatural)

6.2 Chemical contaminants

Parry screens the dry algal meal and the oleoresin for regular testing of pesticides, including dioxins, PCB and PAH and the levels were always below detectable levels. All these analysis were carried out in accredited laboratories. These monitoring forms a part of the quality system followed by Parry.
(Analysis report on pesticides in Parry's AstaNatural – Refer Appendix-3B)
Appendix 3B- Pesticides – AstaNatural – 2.5%
Appendix 3B- Pesticides – AstaNatural – 5%
Appendix 3B- Pesticides – AstaNatural – 7%

6.3 Microbial contaminants

Parry's well monitored HACCP system ensures that strict hygienic practice is followed throughout the system to control microbial contaminants. They include personal hygiene, cleaning, handling and packing procedures. Moreover, the extraction method used by Parry (solvent extraction) also ensures that the microbial load is within specified limits. The current batches show total aerobic count within 1000 which is comparable to other equivalent products. Since the product is an oleoresin, Parry carries out regular analysis of Aflatoxin to ensure the quality.

Table below shows a comparison with equivalent products.

TABLE: 12
Microbiological data comparison

Parameter	AstaNatural	BioAstin	Zanthin
Total aerobic bacteria	<3000	<1000	<1000
Yeast & molds	<100	<100	<100
Coliforms	Negative	<10	NA
E.coli	Negative	Negative	Negative
Salmonella	Negative	Negative	Negative
Staphylococcus	Negative	Negative	Negative

(Appendix-13 Microbiological analysis of AstaNatural and current batch results)

6.4 Toxins

As mentioned earlier, Parry's strict monitoring of parameters such as light intensity, inoculum size, nutrient level and harvesting method ensures that there is no contamination of the culture by other algae such as cyanobacteria or animals. Moreover, Parry tests its products for algal toxins regularly to ensure the safety of the products. We have analysed both archive samples and current production batches for microcystin. Microcystin was not detected in any samples (DL is 0.5 ppb). Microcystin analysis is included as a part of quality check by checking composite production samples once in every two weeks

TABLE: 13
Microcystin analysis Report:

Batch Number	Microcystin Level	Detection Limit
PH-023/07-08	0.5 ppb	0.5 ppb
PH-029/07-08	0.5 ppb	0.5 ppb
PH-034/07-08	0.5 ppb	0.5 ppb
PH-089/08-09	0.5 ppb	0.5 ppb
PH-092/08-09	0.5 ppb	0.5 ppb
PH-078/08-09	0.5 ppb	0.5 ppb
PH-075	0.5 ppb	0.5 ppb
PH-076	0.5 ppb	0.5 ppb
PH-077	0.5 ppb	0.5 ppb

Other algal toxins such as Anatoxin and Cylindrospermopsin were tested periodically by external labs. (Refer Appendix-14). The result shows the levels below the detectable levels thus ensuring the safety of the product (less than 3microgram/litre).

6.5 Solvent residue

Parry produces its astaxanthin oleoresin by solvent extraction process. The multistage process under high vacuum ensures that the residual limit is below the specified limit. Even though the limit is specified as 100ppm, the average value is much below 50ppm (Refer Appendix-3A). This level is much below the prescribed level suggested under the ICH guidelines for residual solvents which is approved in EU(ICH, 1997). Currently we are using and planned to use ethyl acetate as solvent since most of the customers prefer ethyl acetate to Heaxane. Ethyl acetate is a class 3 solvent and permitted daily exposure (PDE) is around 5000 ppm (Q3C [R3]). Ethyl acetate residue in the oleoresin is maintatined below 100 ppm though ICH guidelines is less than 5000 ppm. Moreover considering the fact that the intended use is not more than 4mg, the level of solvent residue in such a small quantity will be negligible. It is well known that commercial carotenoid products such as lutein, zeaxanthin and lycopene are produced by solvent extraction process and has been consumed worldwide for a long time.

It is only the cost which determines or dictates the supplier of these products to adopt certain process and safety wise both are similar.

7. Other relevant data

7.1 Toxicity and Safety studies

Astaxanthin from Haematococcus alga has been used for a longer time in the feed industry to enhance the colour of the flesh of salmon and crustaceans and the skin and yolk of eggs (Davidson, 1993). Astaxanthin has also been regularly used in human diet through the consumption of sea foods such as salmon red fishes, shrimp, krill or lobsters. The alga has been a subject for study since the 19th century. The safety of Haematococcus has been reported by various human and animal studies. The results of these studies indicated no ill effect on the subjects at the administration of Astaxanthin at different dosages (Aqua NIC 2000). The USFDA has approved Astaxanthin from Haematococcus for marketing as a dietary ingredient under the DSHEA and it has also been approved in Japan for use in both foods and animal feeds (FDA, 1999).
The code of federal regulations of the USFDA approves the use of Astaxanthin as a colour additive in salmon feed to a maximum of 80mg / Kg of the total feed.
The studies on toxicity and safety conducted with the alga Haematococcus alga at various dosage levels as high as 18g of algae peg Kg of body weight has revealed no abnormalities for the evaluation on mortality, pharmacotoxic signs and body weights.

In *Haematococcus pluvialis*, astaxanthin occurs as the 3*S*, 3'S stereoisomer, which is the more stable natural form and primarily as monoesters (>90%), with diesters comprising ~8% and the free molecule ~1%. It tends to produce higher pigmentation in rainbow trout compared to synthetic astaxanthin provided at the same dietary concentration . It was noted that the main astaxanthin stereo isomer identified by the FDA researchers in the 5 species of wild Pacific salmon they studied, was the 3S, 3'S stereo isomer, identical to that found in Haematococcus pluvialis.

From various studies it is assumed that usage of 5mg of Astaxanthin / day is considered to be safe for human consumption. A brief description on some of the studies included here.

1. Sub-Acute Toxicity study on Astaxanthin Oleoresin 5% from Parry Nutraceuticals (Appendix-16).

Parry Nutraceuticals conducted toxicity of its product Astaxanthin Oleoresin (AstaNatural 5%) in Albino Wister rat and Albino Swiss mice. The study period was about 30days. Three different doses (250microlitre/kg to 1000microlitre/kg) were given to 12 animals (6 male and 6 female). Study shows Astaxanthin oleoresin causes no local toxicological effects at the site of administration. Haematological analysis revealed no significant abnormalities. Biochemical, species dependent and sex dependent variations were not evidenced.

2. Safety of an Astaxanthin-Rich Haematococcus pluvialis Algal Extract: A Randomized Clinical Trial (Spiller and Dewell, 2003).

A growing body of scientific literature indicates that astaxanthin is a more powerful antioxidant than other carotenoids and vitamin E and may confer numerous health benefits. The purpose of this investigation was to conduct a human safety study with a Haematococcus pluvialis algal extract with high levels of astaxanthin. Thirty-five healthy adults age 35-69 years were enrolled in a randomized, double-blind, placebo-controlled trial of 8 weeks' duration. All participants took three gelcaps per day, one at each meal. Nineteen participants received gelcaps with an algal extract in safflower oil, containing 2 mg of astaxanthin each (treatment); 16 participants received gelcaps containing safflower oil only (placebo). Blood pressure and blood chemistry tests, including a comprehensive metabolic panel and cell blood count, were conducted at the beginning of the trial and after 4 and 8 weeks of supplementation. No significant differences were detected between the treatment and the placebo groups after 8 weeks of supplementation with the algal extract in the parameters analyzed, except for serum calcium, total protein, and eosinophils ($P < .01$). Although the differences in these three parameters were statistically significant, they were very small and are of no clinical importance. These results reveal that 6 mg of astaxanthin per day from a H. pluvialis algal extract can be safely consumed by healthy adults.

3. Effects of Astaxanthin on Larval Growth and Survival of the Giant Tiger Prawn, Penaeus monodon (Darachai et al., 1998).

The aim of this study was to evaluate effects of natural and synthetic astaxanthins on growth, survival and low salinity resistance of Penaeus monodon larvae. Four diets: algal astaxanthin-added diet (AAD), synthetic astaxanthin-added diet (SAD), non astaxanthin supplemented diet (NAD) and natural food (NF) were prepared to feed three P. monodon larval stages (zoea, mysis and postlarvae). The results indicated that zoea fed AAD, NF and NAD survived significantly better than zoea fed SAD. For the mysis stage, larvae fed NF and AAD had better survival rates than those fed NAD and SAD. After 15 days of rearing the postlarval stage to PL-15, AAD showed the best survival rate and it was significantly higher than that of PL-15 that had been fed NF. PL-15 fed AAD and NF were significantly longer than those fed SAD and NAD ($P<0.05$). In low salinity challenge tests, the postlarvae fed AAD showed higher tolerance than postlarvae fed other diets.

4. Rapid liquid chromatographic method to distinguish wild salmon from aquacultured salmon fed synthetic astaxanthin (Turujman et al., 1997).

Analytical methods are needed to determine the presence of color additives in fish. We report a liquid chromatographic (LC) method developed to identify the synthetic form of the color additive astaxanthin in salmon, based on differences in the relative ratios of the configurational isomers of astaxanthin. The distributions of configurational isomers of astaxanthin in the flesh of wild Atlantic and wild Pacific salmon is similar, but significantly different from that in aquacultured salmon. Astaxanthin is extracted from the flesh of salmon, passed through a silica gel Sep-Pak cartridge, and analyzed directly by LC on a Pirkle covalent L-leucine column. No derivatization of the astaxanthin is required-an important advantage of our approach, which is a modification of our previously described method. This method can be used to distinguish between aquacultured and wild salmon. The method has general applicability and can also be used to identify astaxanthins derived from other sources such as Phaffia yeast and Haematococcus pluvialis algae.

5. Chemoprevention of Rat Oral Carcinogenesis by Naturally Occurring Xanthophylls, Astaxanthin and Canthaxanthin (Takuji et al., 1995).

The chemo preventive effects of two xanthophylls, astaxanthin (AX) and canthaxanthin (CX) on oral carcinogenesis induced by 4-nitroquinoline 1-oxide (4-NQO) was investigated in male F344 rats. Rats were given 20 ppm of 4-NQO in their drinking water for 8 weeks to induce oral neoplasms or preneoplasms. Animals were fed diets containing 100 ppm AX or CX during the initiation or postinitiation phase of 4-NQO-induced oral carcinogenesis. The others contained the groups of rats treated with AX or CX alone and untreated. At the end of the study (week 32), the incidences of preneoplastic lesions and neoplasms in the oral cavity of rats treated with 4-NQO and AX or CX were significantly smaller than those of rats given 4-NQO alone ($P < 0.001$). In particular, no oral neoplasms developed in rats fed AX and CX during the 4-NQO exposure and in those given CX after the 4-NQO administration. Similarly, the incidences of oral preneoplastic lesions (hyperplasia and dysplasia) in rats treated with 4-NQO and AX or CX were significantly smaller than that of the 4-NQO-alone group ($P < 0.05$). In addition to such tumor inhibitory potential, dietary exposure of AX or CX decreased cell proliferation activity in the nonlesional squamous epithelium exposed to 4-NQO as revealed by measuring the silver-stained nucleolar organizer regions protein number/nucleus and 5'-bromodeoxyuridine-labeling index. Also, dietary AX and CX could reduce polyamine levels of oral mucosal tissues exposed to 4-NQO. These results indicate that AX and CX are possible chemopreventers for oral carcinogenesis, and such effects may be partly due to suppression of cell proliferation.

6. Suppression of azoxymethane-induced rat colon carcinogenesis by dietary administration of naturally occurring xanthophylls astaxanthin and canthaxanthin during the postinitiation phase (Takuji et al., 1995).

The modulating effects of dietary feeding of two xanthophylls, astaxanthin (AX) and canthaxanthin (CX) during the post initiation phase on colon carcinogenesis initiated with azoxymethane (AOM) were investigated in male F344 rats. Animals were initiated with AOM by weekly s.c. injections of 15 mg/kg body wt for 3 weeks and then they were fed the diets containing AX or CX at concentrations of 100 and 500 ppm for 34 weeks. The others contained the groups of rats treated with AX or CX alone and untreated. At the end of the study (week 37), the incidence and multiplicity of neoplasms (adenoma and adenocarcinoma) in the large intestine of rats initiated with AOM and followed by AX or CX containing diet at a high dose (500 ppm) were significantly smaller than those of rats given AOM alone ($P < 0.001$). In addition, AX or CX feeding significantly inhibited the development of aberrant crypt foci induced by AOM. Dietary exposure to AX or CX also decreased cell proliferation activity as revealed by measuring 5'-bromodeoxyuridine-labeling index in crypt cells, colonic mucosal ornithine decarboxylase activity and blood polyamine levels. These results indicate that AX and CX are possible chemopreventers for carcinogenesis of colon in addition to urinary bladder and oral cavity and such effects may be partly due to suppression of cell proliferation.

7. Growth and survival of Atlantic salmon, Salmon salar L., fed different dietary levels of astaxanthin (Christiansen et al., 1995).

Atlantic salmon fry hatched from pigment-free eggs and from eggs containing the pigment astaxanthin were fed eleven casein/gelatin-based purified diets with varying levels of astaxanthin, ranging from 0 to 317 mg kg1, to determine the optimum dietary astaxanthin level for satisfactory growth and survival during the start-feeding period. The fish were fed the experimental diets for a period of 11 weeks.

No difference in performance was found between the two types of fry originating from the pigment-free eggs and those containing pigment. However, the dietary astaxanthin concentration was found to have a significant effect on both the growth and the survival of fry. Fish fed diets with astaxanthin concentrations below 5.3 mg kg1 were found to have marginal growth. In addition, mortality was high in the groups fed diets with astaxanthin concentrations below 1.0 mg kg1. The specific growth rate (SGR) was also affected by the dietary treatment. The lipid content was higher and the moisture content was lower in the fish fed the diets containing astaxanthin concentrations above 5.3 mg kg1. The vitamin A and astaxanthin concentrations in whole-body samples of the fry were significantly affected by the dietary level of astaxanthin. A plateau level in whole-body vitamin A concentration was observed at dietary levels of approximately 80 mg astaxanthin / kg and higher, while no maximum astaxanthin concentration in whole-body samples was observed within the dietary levels used.

The results suggest the need for a minimum dietary astaxanthin concentration of 5.1 mg kg1 to achieve maximum growth and survival during the start-feeding period. The results indicate a low bioavailability of vitamin A palmitate and acetate and the results also suggest a provitamin A function for astaxanthin during the same period.

8. Carotenoid pigments of the green alga Haematococcus pluvialis: assay on rainbow trout, Oncorhynchus mykiss, pigmentation in comparison with synthetic astaxanthin and canthaxanthin (Georges and Heinrich, 1993).

The green alga Haematococcus pluvialis was found to contain β-carotene (2.2%), astaxanthin (free < 1%, monoester 12.4%, diester 28.8%), canthaxanthin (44.3%) and lutein (11.4%). The total amount of carotenoid was 2.0% of dry algae. Degradation of algal carotenoids occurred during the pelleting process (−3.3%) even at a low temperature (42°C), as well as during 15 days of storage at 7°C ambient temperature (−5.2%).

A feeding trial was conducted to study the effects of dietary algal incorporation on muscle pigmentation of rainbow trout in comparison with synthetic astaxanthin and canthaxanthin alone or mixed (astaxanthin 48% and canthaxanthin 52%). After 4 weeks of feeding, physical colour measurements showed that increased pigmentation of the trout muscle caused an increase in chroma and a reduction in hue and lightness. Muscle of trout fed algae contained 6.2 mg carotenoid/kg versus 12.7 mg/kg for trout fed the mixture of the two synthetic carotenoids, and 11.8 and 10.1 mg/kg for trout fed synthetic astaxanthin and canthaxanthin, respectively. Carotenoid retention in the muscle of trout fed algae was 1.5% which was less than the retention of the carotenoid mixture (3.1%).

9. Utilization of synthetic carotenoids by the prawn Penaeus japonicus reared under laboratory conditions (Genevieve et al., 1993).

Four groups of prawns fed three different pigmented diets (astaxanthin 100 mg/kg, canthaxanthin 100 mg/kg, astaxanthin/canthaxanthin 50/50 mg/kg) and a pigment-free diet were maintained under laboratory rearing conditions during one moulting cycle. Dietary astaxanthin was found to be stored in the integument (carapace and epidermis) and hepatopancreas. Individuals fed the astaxanthin/canthaxanthin mixture showed an accumulation of carotenoids in the epidermis and exhibited the highest survival rate. There was no experimental evidence supporting a possible influence of these pigments on growth under the conditions used in this study.

10. Pigmentation of kuruma prawn, Penaeus japonicus Bate, by various pigment sources and levels and feeding regimes (Yew-Hu and Shu-ching, 1992).

Color is one of the major factors which determine the price of live kuruma prawns, Penaeus japonicus Bate, in the Japanese market. This study was designed to determine the effects of various pigment sources, and levels and feeding regimes on the pigmentation of kuruma prawns.
A 7 × 3 factorial design was used which included seven pigmented diets fed for three time durations with two replications. The seven pigmented diets contained astaxanthin at concentrations of 50, 100 and 200 mg/100 g diet, β-carotene at concentrations of 50, 100 and 200 mg/100 g diet, and algal (Dunaliella salina) meal at a concentration of 100 mg pigment/100 g diet. Prawns were fed each pigmented diet for 1, 2 or 3 months. During the other months, the prawns were fed a non-pigmented diet. The control group was fed a non-pigmented diet exclusively.

Astaxanthin was found to be the most effective pigment. No difference in pigment concentration was found between prawns fed the β-carotene and algal meal. Pigmentation by astaxanthin at 50 mg/ 100 g diet was poorer than at 100 and 200 mg/100 g diet. No significant difference in pigmentation was found among prawns fed the three levels of β-carotene. There was no difference in pigmentation among prawns fed the pigmented diets for the three time periods. The best feeding strategy for pigmentation was to feed the prawns a diet containing astaxanthin at 100 mg/100 g for 1 month before harvest. Prawns fed the astaxanthin diet had a higher rate of survival than those fed the β-carotene or algal meal diets. A positive correlation between survival rates and pigment concentration in prawn tissue indicated that pigment may play a role in improving the survival of prawns.

11. Pigmentation of adult rainbow trout, Oncorhynchus mykiss, using the green alga Haematococcus pluvialis (Sommer et al., 1992).

A feeding trial was conducted to assess the effects of supplementing the diets of commercial-size trout with carotenoid-rich microalgae. The flesh colour scores, total carotenoid and astaxanthin levels in the flesh increased throughout the trial in groups receiving pigment supplements. Regression analyses demonstrated that the total carotenoid and astaxanthin levels were significantly related to the level of algal pigment in the diet (0, 20, 40, 60, 80 mg total carotenoid/kg feed). Analyses of total carotenoid in trout skin at the end of the trial (day 100) showed a similar trend. Addition of natural or synthetic pigment to the diet had a near significant effect of growth ($p<0.07$). Microalgal supplements appeared to have no effect on survival. Algal pigment and synthetic astaxanthin (Roche carophyll pink) were compared at a similar inclusion rate (80 mg total carotenoid/kg feed). Synthetic astaxanthin resulted in significantly higher levels of total carotenoid and astaxanthin in the flesh. The algal pigment resulted in the deposition of small, but statistically significant, quantities of adinorubin. Possible causes for these differences are discussed.

7.2 Quality and Hygiene system

As mentioned earlier, Parry adopts a stringent Quality and Hygiene system in its production unit. Parry has ISO 9000 (2000), cGMP, HACCP and ISO 14000 certificates under its umbrella. Parry has a thorough vendor verification program, strict monitoring of raw materials used in cultivation and processing, quality checks and approval before the star of each process, In-process quality checks and approval of every batch when it is produced and dispatched. Dispatch section ensures that the product reaches the customers in a safe mode. A corrective and preventive program ensures that the deviations are kept at a minimal level.

Hygienic program under HACCP system ensures hygienic code is followed in each and every step of the process and also in the final product. Thus Good Manufacturing Process is followed and monitored regularly to ensure that the product is safe and retains the quality when it reaches the customer.

8. Conclusion

The information provided above - in particular the results of toxicity studies, heavy metal analysis and low solvent residues in the product indicate that Parry's AstaNatural from Haematococcus pluvialis is a safe and hygienic product. It can be concluded that ingestion of Parry's AstaNatural at the suggested daily intake of not more than 4 mg Astaxanthin would not lead to any adverse effects in humans in the short or long term. Moreover, from the data provided it is clear that Parry's AstaNatural is similar to BioAstin. Hence, Parry request its application on substantial equivalence to the existing BioAstin to be considered in the context of Article 5 of EC regulation No. 258/97.

9. Appendices

Appendix-1. Flow chart on cultivation, harvesting and drying of Haematococcus cell powder (CONFIDENTIAL).

Appendix-2. The process flow chart and preparation of Astaxanthin oleoresin (CONFIDENTIAL).

Appendix-3A. General composition of AstaNatural.
Appendix-3B – Pesticies data of AstaNatural 2.5% 5% 7%

Appendix-4. Total Astaxanthin percentage in AstaNatural

Appendix-5. Determination of Total astaxanthin in AstaNatural by spectroscopic method.

Appendix-6. Determination of Astaxanthin by HPLC method

Appendix-7. Astaxanthin profile of Haematococcus cell powder and AstaNatural oleresins)

Appendix-8. Fatty acid profile of Haematococcus cell powder and AstaNatural oleresins)

Appendix-9A - Specification sheet of Parry's Haematococcus cell powder
Appendix-9B - Specification sheet of Parry's AstaNatural – 2.5%
Appendix-9C - Specification sheet of Parry's AstaNatural – 5%
Appendix-9D Specification sheet of Parry's AstaNatural – 7%

Appendix-10. General composition of Parry's Haematococcus cell powder – average of three lots

Appendix-11. Stability data - AstaNatural

Appendix -12. Heavy metal analysis report of AstaNatural

Appendix-13. Microbiological analysis of AstaNatural

Appendix-14. Analysis of algal toxins.

Appendix-16. Report on Sub-Acute toxicity study on Astaxanthin Oleoresin 5% from Parry Nutraceuticals.

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