Technical report

Haematococcus pluvialis algal meal produced with Aquasearch's proprietary technology: a unique source of natural astaxanthin and algal nutrients.

(TR.3004.001)

- ***Haematococcus pluvialis** is an ubiquitous, unicellular green alga that produces the carotenoid pigment, astaxanthin, when in the resting, or "haematocyst", stage.*
- ***To produce the meal, the green, swimming stages of H. pluvialis are cultivated in closed, tubular growth modules. When at sufficient concentrations, green cells are transferred to ponds where conditions favor the rapid transition into the red, resting stage.***
- ***Red haematocysts are harvested, dewatered, and mechanically broken to improve the bioavailability of astaxanthin. The resulting slurry is pasteurized and dried at moderate temperature into flakes which are ground up and mixed with a natural food grade antioxidant to stabilize the pigment. The algal meal is then packed into aluminum foil-lined polyethylene bags.***
- ***Haematococcus pluvialis algal meal is at least 2% astaxanthin, of which >80% is in the monoester form. The meal typically contains >18% protein and >19% fat, and has a low bacterial counts within standard human safety levels.***

1. Occurrence and life cycle of *Haematococcus pluvialis*.

Haematococcus pluvialis **Flotow, 1844**
Family: Haematococcaceae
Order: Volvocales
Class: Chlorophyceae
Division: Chlorophyta

Haematococcus pluvialis is a flagellated unicellular green alga (Chlorophyte) that produces the red pigment, astaxanthin, during a resting or encysted stage induced by extreme environmental conditions. *H. pluvialis* was described in 1844, although what may be synonymous species were recognized much earlier (Table 1). It is widely distributed across Europe, Africa and North America, typically in small, ephemeral pools (Almgren 1966, Thompson & Wujek 1989). The striking red color it imparts to such pools led to extensive study, which continues to the present,

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of its natural history and production of red "haematochrome" (now known to be astaxanthin), which continues to the present.

H. pluvialis changes in morphology during a life cycle that is driven by environmental conditions (Elliot 1934, Droop 1953, 1954; Almgren 1966):

a) The large (8-50 μm diameter), flagellated green form or **macrozooid** is the most common in rapidly growing cultures. Macrozooids divide into other macrozooids, usually in the early morning.
b) As conditions deteriorate (e.g., slow desiccation, extremes in pH and strong illumination), macrozooids lose their flagella and become highly vacuolated, floating **palmellae** which remain green. Palmellae can divide into other palmellae, or into flagellated macrozooids.
c) Under nutrient-poor conditions, red **haematocysts** typically develop from palmellae that have ceased to divide, but occasionally may arise from macrozooids. Haematocysts have heavy cell walls and produce large amounts of astaxanthin, when in bright sunlight.
d) If conditions improve, haematocysts divide into small (~20 μm), cylindrical flagellated **microzooids** which eventually lose their flagella, enlarge, and become palmellae. More often, however, haematocysts divide into larger macrozooids and the cycle repeats.

Aquasearch has several existing and pending patents for its unique proprietary technology to grow and process *Haematococcus* algae. This proprietary technology ensures the highest quality of algal meal as a source of natural astaxanthin and other nutrients for use in dietary supplements.

2. Culturing *Haematococcus* algae (Fig. 1)

The life cycle of *Haematococcus* algae dictates how production of natural astaxanthin is optimized. At Aquasearch , Inc., the algae are grown in three phases:

a) Small-volume stock cultures maintained in the laboratory are scaled up in increasingly larger containers to produce inoculum for outdoor mass culture;
b) Continuous culture of macrozooids in large, tubular closed photobioreactors or modules under nutrient-replete conditions;
c) Reddening in ponds under nutrient-poor conditions.

In the first two phases, the emphasis is on obtaining high concentrations of *Haematococcus* over very short periods of time. Rapid cell proliferation is maintained in Aquasearch Growth Modules (AGMs) by periodically harvesting part of the culture into ponds and replacing this with fresh nutrient medium. In ponds, on the other hand, conditions are controlled to accelerate cell encystment and astaxanthin production. The ability to grow large concentrations of pure cultures of *Haematococcus pluvialis* in AGMs and to stock ponds with those high concentrations ensures a very rapid and synchronized reddening, minimizing the risk of contamination and maximizing astaxanthin production, while minimizing levels of astaxanthin intermediary metabolites and other carotenoids.

3. Processing of algal meal (Fig. 1)

When a pond culture has properly reddened, it is harvested, dewatered and rinsed. Cells in the resulting slurry are then mechanically broken to improve the release of the pigment. This process

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improves bioavailability of astaxanthin when ingested, since intact cyst walls are inefficiently digested (Sommer et al., 1994). This cell wall rupture process also helps deactivate other potentially occurring harmful micro-organisms. Cell-broken product is subsequently pasteurized and dried at moderate temperature to minimize degradation of astaxanthin and other nutrients, while reducing bacterial counts to levels safe for human consumption (Table 2). Because the product results from a natural growth process and is subject to natural variability, several drying batches may be subsequently mixed in order to achieve predetermined standard astaxanthin levels: 2%, 2.5% and 3%. The resulting dry flakes of one or several batches are then ground up to a powder and mixed with an approved food-grade natural antioxidant, which helps stabilize the pigment during storage. The algal meal is then packed in air-tight aluminum-lined polyethylene bags and stored until further processing.

4. Algal meal composition (Table 2)

Haematococcus pluvialis algal meal is a good source of protein, fat, astaxanthin and other micronutrients. It accumulates astaxanthin when stressed by environmental changes (Grung et al. 1992). The astaxanthin stereo-isomer deposited in *Haematococcus pluvialis* is the 3*S*,3'*S* form (Grung et al. 1992), which is the main stereo-isomer encountered in wild salmon and many other aquatic species (Turujman 1997, Aquasearch 1999a). Typically, the main form of astaxanthin accumulated in *H. pluvialis* is the esterified form: monoesters represent more than 80% of the total astaxanthin, while diesters represent approximately 10 to 15%. Small amount of free astaxanthin (less than 10% of total astaxanthin) are also found (Akvaforsk, 1999, Latasa, 1995). In addition to astaxanthin other carotenoids can be encountered in *Haematococcus pluvialis*. Recent analyses on a representative batch determined that astaxanthin represented 84% of total carotenoids (Akvaforsk, 1999). This confirms analyses by other investigators (Table 3), with astaxanthin usually representing more than 85% of total carotenoids. Other carotenoids (Table 3) detected in small amounts in *Haematoccus* algae include: beta-carotene (0 to 5%), lutein (1 to 11%), cantaxanthin (2 to 5%). Analyses of Aquasearch algal meal have detected mainly lutein and beta-carotene (Latasa, 1995), although up to 2% of total carotenoids may be occurring as cantaxanthin (Aquasearch 1999). Because the process control and proprietary production technology used by Aquasearch is aimed at maximising astaxanthin production, the level of other carotenoid pigments stays within 10 to 15% of total carotenoids content.
Haematococcus has been employed as a constituent of feeds for a variety of animals, with no negative effects observed on growth, survival, behaviour, physiology or biochemistry (Nakazoe et al. 1984; Schiedt et al. 1986; Storrebakken et al. 1987; Ito et al. 1989; Sommer et al. 1991, 1992; Choubert & Heinrich 1993). *Haematococcus pluvialis* has been fed at levels up to 6% in trout diets without any negative effects (Choubert & Heinrich 1993). There is no indication that *Haematococcus* spp. contain any chemical compounds that may have a deleterious effect on other organisms. Safety studies conducted by Aquasearch in a representative animal model and with human volunteers have demonstrated that astaxanthin dietary supplements formulated with *Haematococcus pluvialis*, grown and processed with Aquasearch proprietary technology, are safe for human consumption, when following suggested inclusion levels (Aquasearch, 1999b).

5. References

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Table 1. Possible synonyms for *Haematococcus pluvialis* Flotow, 1844 (Almgren, 1966).

Scientific names	Dates
Volvox lacustris Girod-Chantrans	1802
Lepraria kermesina Wrangel	1824
Sphaerella Wranglelii Sommerfelt	1824
Protococcus nivalis Agardh em. Greville	1824
Chlorococcum kermesinum (Wrangel) Fries	1825
Byssus kermesina (Wrangel) Wahlenberg	1826
Haematococcus noltii Agardh	1828
Haematococcus Grevillei Agardh	1828
Protococcus monospermus Gorda	1833
Microcystis grevillei (Agardh) Kutzing	1833
Globulina kermesina (Wrangel) Turpin	1836
Discerea purpurea A. et C. Morren	1841
Protococcus cordae Meneghini	1843
Haematococcus pluvialis Flotow	1844
Protosphaera pluvialis (Flotow) Trevisan	1848
Protosphaera cordae (Meneghini) Trevisan	1848
Protococcus pluvialis (Flotow) Kutzing	1849
Chlamydococcus pluvialis (Flotow) Braun	1852
Hysginum pluviale (Flotow) Perty	1852
Haematococcus lacustris (Girod-Chantrans) Rostafinski	1875
Sphaerella pluvialis (Flotow) Wittrock	1896
Sphaerella lacustris (Girod-Chantrans) Wittrock	1896

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Fig. 1. Aquasearch's production process for the algal meal prepared from the green algae: *Haematococcus pluvialis*.

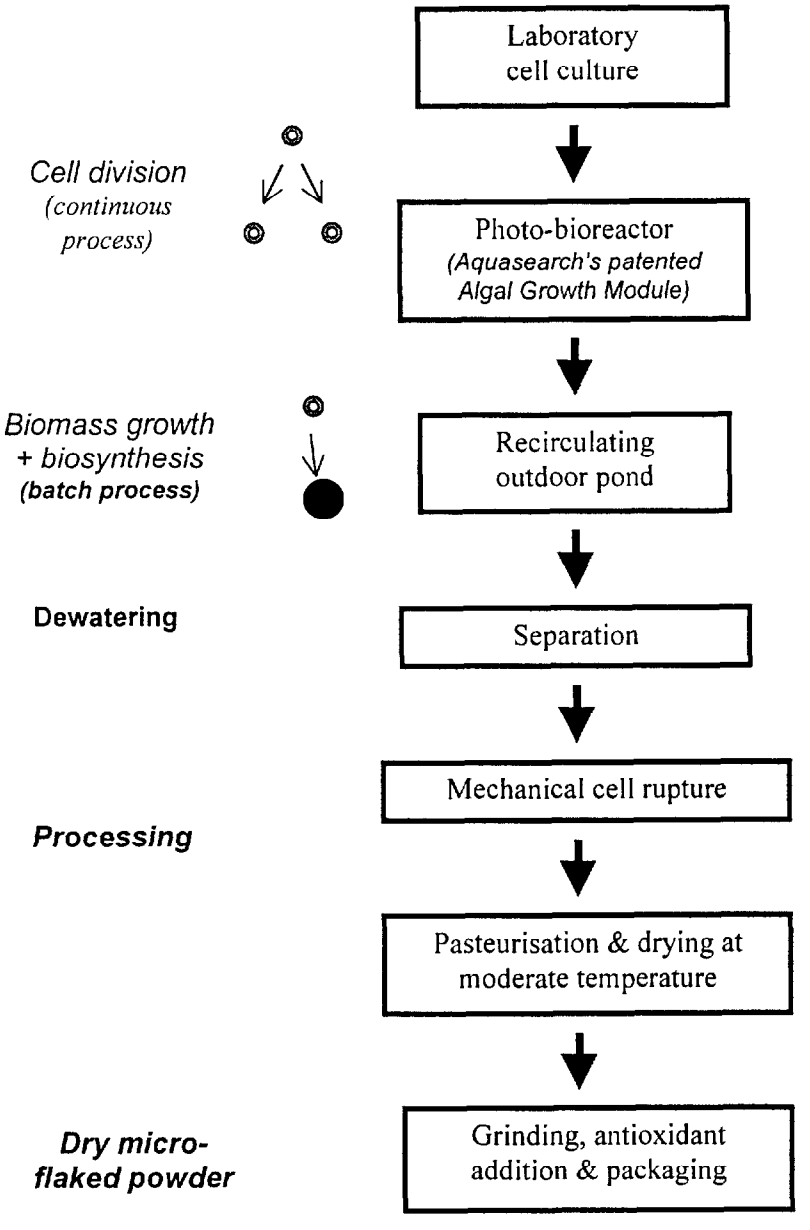

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Table 2. Typical composition of Aquasearch's dried *Haematococcus pluvialis* algal meal.

Proximate analysis

Crude protein	18.08	%
Crude fat	19.43	%
Ash	3.28	%
Crude fiber	4.30	%
Moisture	3.54	%

Energy

Calories	470 per 100 g
Calories from fat	175 per 100 g
Calories from Saturated fat	28 per 100 g

Carbohydrates

Dietary fiber	**26.5**	**%**
Insoluble fiber	*25.2*	*%*
Soluble fiber	*1.3*	*%*
Sugars	**0.77**	**%**
Cholesterol	**0**	**%**

Amino acids

Alanine	7.48	%
Arginine	5.54	%
Aspartic acid	7.62	%
Cystine/Cysteine	0.90	%
Glutamic acid	9.60	%
Glycine	5.01	%
Histidine	1.52	%
Isoleucine	3.55	%
Leucine	7.73	%
Lysine	4.33	%
Methionine	1.5	%
Phenylalanine	3.56	%
Proline	4.83	%
Serine	4.77	%
Threonine	4.99	%
Tryptophan	1.60	%
Tyrosine	2.92	%
Valine	5.28	%
Met + Cys	2.39	%
Met + tyr	6.48	%

Micro-organisms

Aerobic plate count	< 1000 CFU/g
E. coli	< 10 CFU/g
Salmonella	Negative/25 g

Heavy metals

Lead	<0.5	ppm
Mercury	<0.1	ppm
Cadmium	<0.5	ppm
Arsenic	<0.5	ppm

Carotenoids

Total carotenoids	2.6	%
Total astaxanthin	2.18	%
Free astaxanthin	*3.6*	*%*
Astaxanthin monoester	*81.8*	*%*
Astaxanthin diester	*14.6*	*%*

Fatty acids

Caprylic acid	C-8:0	<0.01 %
Capric acid	C-10:0	<0.01 %
Lauric	C-12:0	<0.01 %
Myristic	C-14:0	0.09 %
	C-14:1	<0.01 %
Palmitic	C-16:0	2.82 %
Palmitoleic	C-16:1	0.11 %
Stearic	C-18:0	0.17 %
Oleic	C-18:1	3.28 %
Linoleic	C-18:2	3.11 %
γ linolenic ω-6	C-18:3	1.89 %
Octadecatetraenoic	C-20:0	0.04 %
Gadoleic	C-20:1	0.03 %

Total saturated fat:	**3.12 %**
Tot.monosaturated fat:	**3.42 %**
Tot.polyunsaturated fat:	**5.00 %**

Vitamins

Vitamin A	22000	IU/100 g
Alpha tocopherol	412	mcg/g*
Vitamin B6	0.14	mg/100 g
Vitamin B12	0.04	mg/100 g
Thiamine (B1)	0.09	mg/100 g
Riboflavin (B2)	0.26	mg/100 g
Niacin	0.45	mg/100 g
Folic acid	0.39	mg/100 g
Pantothenic acid	2.47	mg/100 g
Vitamin C	0	mg/100 g

(* before addition of any antioxidant)

Minerals

Calcium	890	ppm
Phosphorous	3900	ppm
Potassium	2300	ppm
Sodium	2400	ppm
Magnesium	1500	ppm
Iron	880	ppm
Cobalt	0.58	ppm
Nickel	5.7	ppm
Selenium	<0.5	ppm
Molybdenum	<0.5	ppm
Zinc	49	ppm
Chromium	5.1	ppm

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Table 3. Carotenoids content and distribution in *Haematococcus pluvialis* cysts

	Individual carotenoid pigments measured as % of total carotenoids									
Sources:	A	B	C	D	E	F	G	H*	K*	**Range**
Carotenoid pigments										
Astaxanthin (total)	89.0	84.7	>79.2	84-89	81.0	81.5	85.0	95.4	83.8	**81 – 95**
Free astaxanthin	*1.0*	*n.a.*	*n.a.*		*1.0*	*n.a.*	*5.0*	*2.4*	*2.06*	***1-5***
Astaxanthin monoesters	*49.0*	*n.a.*	*79.2*	*69-74*	*46.0*	*n.a.*	*70.0*	*83.2*	*68.6*	***46-79***
Astaxanthin diesters	*39.0*	*n.a.*	*n.a.*	*10-20*	*34.0*	*n.a.*	*10.0*	*9.8*	*12.2*	***10-39***
Beta-carotene	3.0	<1.3	*n.a.*		5.0		*n.a.*		*n.a.*	**0-5**
Lutein	1.0	9.8	*n.a.*	1-9	6.0	10.6	*n.a.*	3.6	*n.a.*	**1-11**
Cantaxanthin	2.0	5.5	*n.a.*		4.0		*n.a.*		*n.a.*	**0-5.5**
Other carotenoids	5.0	<1.3	*n.a.*	6-9	4.0		*n.a.*	0.9	*n.a.*	**1-9**

Sources: A = Harker & Young (1995), B = Fan et al. (1995), C = Yuan et al. (1996), D = Koyabashi et al. (1991), E = Grung et al. (1992), F = Zlotnik et al. (1993), G = Lorenz (1998), H = Latasa (1995), I = Akvaforsk (1999)
** = Aquasearch's Haematococcus algal meal. N.a. = not analyzed.*

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NUTRAXAN™ ASTA

Dried *Haematococcus pluvialis* algal meal

DESCRIPTION AND USE:

NUTRAXAN™ASTA is a dried algal meal prepared from *Haematococcus pluvialis* algae. After harvest, the algae are cell-broken, gently dried, stabilised with a natural antioxidant, and packed in heat-sealed, air-tight, aluminum-lined, polyethylene bags. During the processing they also undergo a pasteurization step to maintain a low bacterial count. NUTRAXAN™ASTA is a very good source of **natural astaxanthin, a superior biological antioxidant**, and of other micro-algal nutrients. Several grades of NUTRAXAN™ASTA are available, based on the guaranteed total astaxanthin content: Minimum 2.0%, 2.5% or 3.0%.

NUTRAXAN™ASTA is to be used as an ingredient for dietary supplements. Recommended inclusion rate: 250 mg or 5 mg astaxanthin per daily serving.

NUTRAXAN™ASTA has been produced under Aquasearch's strict manufacturing standards and proprietary technology for algae culture and processing, aimed at maximising nutrient content and availability, while ensuring that potential contaminants hazardous to health are maintained below accepted safe levels.

TYPICAL COMPOSITION

Proximate analysis		
Crude protein	18.08	%
Crude fat	19.43	%
Ash	3.28	%
Crude fiber	4.30	%
Moisture	3.54	%

Carotenoids		
Total carotenoids	2.6	%
Total astaxanthin	2.18	%
Free astaxanthin	*3.6*	%
Astaxanthin monoester	*87.2*	%
Astaxanthin diester	*14.6*	%

Energy	
Calories	470 per 100 g
Calories from fat	175 per 100 g
Calories from saturated fat	28 per 100 g
Carbohydrates	55.67 %
Dietary fiber	**26.5** %
Insoluble fiber	*25.2* %
Soluble fiber	*1.3* %
Sugars	**0.77** %
Cholesterol	**0** %

Fatty acids		
Caprylic acid	C-8:0	<0.01 %
Capric acid	C-10:0	<0.01 %
Lauric	C-12:0	<0.01 %
Myristic	C-14:0	0.09 %
Myristoleic	C-14:1	<0.01 %
Palmitic	C-16:0	2.82 %
Palmitoleic	C-16:1	0.11 %
Stearic	C-18:0	0.17 %
Oleic	C-18:1	3.28 %
Linoleic	C-18:2	3.11 %
γ linolenic ω-6	C-18:3	1.89 %
Octadecatetraenoic	C-20:0	0.04 %
Gadoleic	C-20:1	0.03 %

Total saturated fat:	**3.12 %**
Tot.monosaturated fat:	**3.42 %**
Tot.polyunsaturated fat:	**5.00 %**

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Amino acids

Alanine	7.48	%
Arginine	5.54	%
Aspartic acid	7.62	%
Cystine/Cysteine	0.90	%
Glutamic acid	9.60	%
Glycine	5.01	%
Histidine	1.52	%
Isoleucine	3.55	%
Leucine	7.73	%
Lysine	4.33	%
Methionine	1.50	%
Phenylalanine	3.56	%
Proline	4.83	%
Serine	4.77	%
Threonine	4.99	%
Tryptophan	1.60	%
Tyrosine	2.92	%
Valine	5.28	%
Met + Cys	2.39	%
Met + Tyr	6.48	%

Minerals

Calcium	890	ppm
Phosphorous	3900	ppm
Potassium	2300	ppm
Sodium	2400	ppm
Magnesium	1500	ppm
Iron	880	ppm
Cobalt	0.58	ppm
Nickel	5.7	ppm
Selenium	<0.5	ppm
Molybdenum	<0.5	ppm
Zinc	49	ppm
Chromium	5.1	ppm

Vitamins

Vitamin A	22000	IU/100 g
Alpha tocopherol	412	mcg/g*
Vitamin B6	0.14	mg/100 g
Vitamin B12	0.04	mg/100 g
Thiamine (B1)	0.09	mg/100 g
Riboflavin (B2)	0.26	mg/100 g
Niacin	0.45	mg/100 g
Folic acid	0.39	mg/100 g
Pantothenic acid	2.47	mg/100 g
Vitamin C	0	mg/100 g

(before addition of any antioxidant)*

Heavy metals

Lead	<0.5	ppm
Mercury	<0.1	ppm
Cadmium	<0.5	ppm
Arsenic	<0.5	ppm

Microorganisms

Aerobic plate count	< 1000	CFU/g
E. coli	< 10	CFU/g
Salmonella	Negative/25 g	

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NUTRAXAN™ASTA

Ingredient for dietary supplements.
Source of natural astaxanthin, biological antioxidant.

Net weight: 10-kg Lot #:
Manufacturing date:
Use by: when stored unopened at 20°C
Manufactured by:
AQUASEARCH, INC., Kona Production Facility,73-4460 Queen Kaahumanu Hwy., Suite 110, Kailua-Kona, HI 96740, USA.

Supplement facts

Daily serving size:	250	mg	
Servings per bag:	40,000		
Amount per serving:		Unit	% daily value
Total calories	1.17	cal	0%
Total fat	48	mg	0%
Polyunsaturated fat	12	mg	n.a.
Sodium	1.3	mg	0%
Total carbohydrate	138	mg	0%
Protein	45	mg	0%
Vitamin A	55	IU	1%
Alpha-tocopherol	0.1(0.9)*	mg	1% (9%)*
Total carotenoids	6	mg	n.a.**
Total natural astaxanthin	**5**	**mg**	n.a.**

Ingredients:
Algal meal (*Haematococcus pluvialis*), natural antioxidant (natural vitamin E and/or grapeseed extract).
** 9% if natural Vitamin E added as antioxidant, 1% if no Vitamin E added .*
*** n.a.= not applicable.*

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Dried *Haematococcus pluvialis* algal meal

DESCRIPTION AND USE:

Aquasearch's **Dried *Haematococcus pluvialis* algal meal** is prepared from *Haematococcus pluvialis* algae. After harvest, the algae are cell-broken, gently dried, stabilised with a natural antioxidant, and packed in heat-sealed, air-tight, aluminum-lined, polyethylene bags. During the processing they also undergo a pasteurisation step to maintain a low bacterial count. **Dried *Haematococcus pluvialis* algal meal** is a very good source of **natural astaxanthin, a superior biological antioxidant**, and of other micro-algal nutrients. Several grades of **Dried *Haematococcus pluvialis* algal meal** are available, based on the guaranteed total astaxanthin content: Minimum 2.0%, 2.5% or 3.0%.

Dried *Haematococcus pluvialis* algal meal is to be used as an ingredient for dietary supplements. Recommended inclusion rate: 250 mg or 5 mg astaxanthin per daily serving.

Dried *Haematococcus pluvialis* algal meal has been produced under Aquasearch's strict manufacturing standards and proprietary technology for algae culture and processing, aimed at maximizing nutrient content and availability, while ensuring that potential contaminants hazardous to health are maintained below accepted safe levels.

TYPICAL COMPOSITION

Proximate analysis

Crude protein	18.08	%
Crude fat	19.43	%
Ash	3.28	%
Crude fiber	4.30	%
Moisture	3.54	%

Carotenoids

Total carotenoids	2.6	%
Total astaxanthin	2.18	%
Free astaxanthin	*3.6*	%
Astaxanthin monoester	*87.2*	%
Astaxanthin diester	*14.6*	%

Energy

Calories	470 per 100 g	
Calories from fat	175 per 100 g	
Calories from saturated fat	28 per 100 g	
Carbohydrates	55.67	%
Dietary fiber	**26.5**	%
Insoluble fiber	*25.2*	%
Soluble fiber	*1.3*	%
Sugars	**0.77**	%
Cholesterol	**0**	%

Fatty acids

Caprylic acid	C-8:0	<0.01 %
Capric acid	C-10:0	<0.01 %
Lauric	C-12:0	<0.01 %
Myristic	C-14:0	0.09 %
Myristoleic	C-14:1	<0.01 %
Palmitic	C-16:0	2.82 %
Palmitoleic	C-16:1	0.11 %
Stearic	C-18:0	0.17 %
Oleic	C-18:1	3.28 %
Linoleic	C-18:2	3.11 %
γ linolenic ω-6	C-18:3	1.89 %
Octadecatetraenoic	C-20:0	0.04 %
Gadoleic	C-20:1	0.03 %

Total saturated fat:	**3.12 %**
Tot.monosaturated fat:	**3.42 %**
Tot.polyunsaturated fat:	**5.00 %**

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Suite 110, Kailua-Kona, HI 96740, USA,

DATASHEET

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Amino acids

Alanine	7.48	%
Arginine	5.54	%
Aspartic acid	7.62	%
Cystine/Cysteine	0.90	%
Glutamic acid	9.60	%
Glycine	5.01	%
Histidine	1.52	%
Isoleucine	3.55	%
Leucine	7.73	%
Lysine	4.33	%
Methionine	1.50	%
Phenylalanine	3.56	%
Proline	4.83	%
Serine	4.77	%
Threonine	4.99	%
Tryptophan	1.60	%
Tyrosine	2.92	%
Valine	5.28	%
Met + Cys	2.39	%
Met + Tyr	6.48	%

Minerals

Calcium	890	ppm
Phosphorous	3900	ppm
Potassium	2300	ppm
Sodium	2400	ppm
Magnesium	1500	ppm
Iron	880	ppm
Cobalt	0.58	ppm
Nickel	5.7	ppm
Selenium	<0.5	ppm
Molybdenum	<0.5	ppm
Zinc	49	ppm
Chromium	5.1	ppm

Vitamins

Vitamin A	22000	IU/100 g
Alpha tocopherol	412	mcg/g*
Vitamin B6	0.14	mg/100 g
Vitamin B12	0.04	mg/100 g
Thiamine (B1)	0.09	mg/100 g
Riboflavin (B2)	0.26	mg/100 g
Niacin	0.45	mg/100 g
Folic acid	0.39	mg/100 g
Pantothenic acid	2.47	mg/100 g
Vitamin C	0	mg/100 g

(before addition of any antioxidant)*

Heavy metals

Lead	<0.5	ppm
Mercury	<0.1	ppm
Cadmium	<0.5	ppm
Arsenic	<0.5	ppm

Microorganisms

Aerobic plate count	< 1000	CFU/g
E. coli	< 10	CFU/g
Salmonella	Negative/25 g	

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Haematococcus pluvialis algal meal

Ingredient for dietary supplements.
Source of natural astaxanthin, biological antioxidant.

Net weight: 10-kg Lot #:
Manufacturing date:
Use by: when stored unopened at 20°C
Manufactured by:
AQUASEARCH, INC., Kona Production Facility,73-4460 Queen Kaahumanu Hwy., Suite 110, Kailua-Kona, HI 96740, USA.

Supplement facts

Daily serving size:	250	mg	
Servings per bag:	40,000		
Amount per serving:		Unit	% daily value
Total calories	1.17	cal	0%
Total fat	48	mg	0%
Polyunsaturated fat	12	mg	n.a.
Sodium	1.3	mg	0%
Total carbohydrate	138	mg	0%
Protein	45	mg	0%
Vitamin A	55	IU	1%
Alpha-tocopherol	0.1 (0.9)*	mg	1% (9%)*
Total carotenoids	6	mg	n.a.*
Total natural astaxanthin	**5**	**mg**	n.a.*

Ingredients:
Algal meal (*Haematococcus pluvialis*), natural antioxidant (natural vitamin E and/or grapeseed extract).
** 9% if natural Vitamin E added as antioxidant, 1% if no Vitamin E added .*
*** n.a.= not applicable.*

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LBLam, 12/06/99

DATASHEET

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The AstaFactor™ algal meal

Dried *Haematococcus pluvialis* algal meal

DESCRIPTION AND USE:

The AstaFactor™ algal meal is a dried algal meal prepared from *Haematococcus pluvialis* algae. After harvest, the algae are cell-broken, gently dried, stabilised with a natural antioxidant, and packed in heat-sealed, air-tight, aluminum-lined, polyethylene bags. During the processing they also undergo a pasteurisation step to maintain a low bacterial count. **The AstaFactor™ algal meal** is a very good source of **natural astaxanthin, a superior biological antioxidant**, and of other micro-algal nutrients. Several grades of The AstaFactor™ algal meal are available, based on the guaranteed total astaxanthin content: Minimum 2.0%, 2.5% or 3.0%.

The AstaFactor™ algal meal is to be used as an ingredient for dietary supplements. Recommended inclusion rate: 250 mg or 5 mg astaxanthin per daily serving.

The AstaFactor™ algal meal has been produced under Aquasearch's strict manufacturing standards and proprietary technology for algae culture and processing, aimed at maximising nutrient content and availability, while ensuring that potential contaminants hazardous to health are maintained below accepted safe levels.

TYPICAL COMPOSITION

Proximate analysis		
Crude protein	18.08	%
Crude fat	19.43	%
Ash	3.28	%
Crude fiber	4.30	%
Moisture	3.54	%

Carotenoids		
Total carotenoids	2.6	%
Total astaxanthin	2.18	%
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Energy	
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Calories from fat	175 per 100 g
Calories from Saturated fat	28 per 100 g
Carbohydrates	55.67 %
Dietary fiber	**26.5 %**
Insoluble fiber	*25.2 %*
Soluble fiber	*1.3 %*
Sugars	**0.77 %**
Cholesterol	**0 %**

Fatty acids		
Caprylic acid	C-8:0	<0.01 %
Capric acid	C-10:0	<0.01 %
Lauric	C-12:0	<0.01 %
Myristic	C-14:0	0.09 %
Myristoleic	C-14:1	<0.01 %
Palmitic	C-16:0	2.82 %
Palmitoleic	C-16:1	0.11 %
Stearic	C-18:0	0.17 %
Oleic	C-18:1	3.28 %
Linoleic	C-18:2	3.11 %
γ linolenic ω-6	C-18:3	1.89 %
Octadecatetraenoic	C-20:0	0.04 %
Gadoleic	C-20:1	0.03 %
Total saturated fat:		**3.12 %**
Tot.monosaturated fat:		**3.42 %**
Tot.polyunsaturated fat:		**5.00 %**

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DATASHEET

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Lysine	4.33	%
Methionine	1.50	%
Phenylalanine	3.56	%
Proline	4.83	%
Serine	4.77	%
Threonine	4.99	%
Tryptophan	1.60	%
Tyrosine	2.92	%
Valine	5.28	%
Met + Cys	2.39	%
Met + Tyr	6.48	%

Minerals

Calcium	890	ppm
Phosphorous	3900	ppm
Potassium	2300	ppm
Sodium	2400	ppm
Magnesium	1500	ppm
Iron	880	ppm
Cobalt	0.58	ppm
Nickel	5.7	ppm
Selenium	<0.5	ppm
Molybdenum	<0.5	ppm
Zinc	49	ppm
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Vitamins

Vitamin A	22000	IU/100 g
Alpha tocopherol	412	mcg/g*
Vitamin B6	0.14	mg/100 g
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Niacin	0.45	mg/100 g
Folic acid	0.39	mg/100 g
Pantothenic acid	2.47	mg/100 g
Vitamin C	0	mg/100 g

(before addition of any antioxidant)*

Heavy metals

Lead	<0.5	ppm
Mercury	<0.1	ppm
Cadmium	<0.5	ppm
Arsenic	<0.5	ppm

Microorganisms

Aerobic plate count	< 1000	CFU/g
E. coli	< 10	CFU/g
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The AstaFactor algal meal

Ingredient for dietary supplements.
Source of natural astaxanthin, biological antioxidant.

Net weight: 10-kg Lot #:
Manufacturing date:
Use by: when stored unopened at 20°C
Manufactured by:
AQUASEARCH, INC., Kona Production Facility,73-4460 Queen Kaahumanu Hwy., Suite 110, Kailua-Kona, HI 96740, USA.

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Sodium	1.3	mg	0%
Total carbohydrate	138	mg	0%
Protein	45	mg	0%
Vitamin A	55	IU	1%
Alpha-tocopherol	0.9	mg	9%
Total carotenoids	6	mg	n.a.*
Total natural astaxanthin	**5**	**mg**	n.a.*

Ingredients:
Algal meal (*Haematococcus pluvialis*), natural antioxidant (natural vitamin E).
* n.a.= not applicable.

LBLAFam, 12/06/99

Office of Special Nutritionals (HFS-450)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C Street SW
Washington DC 20205

Aquasearch Inc.
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WebSite: http://www.aquasearch.com/
E-mail: aqse@aquasearch.com

December 6, 1999

Re: New Dietary Ingredient Notification for *Haematococcus* algal meal. Stability studies

Dear Administrator,

As part of our New Dietary Ingredient Notification for *Haematococcus* algal meal, we have started stability studies (see section 7), which will support the shelf-life and "use by" recommendations that will appear on the label (see section 2).

Before the end of the 75-day period after notification we will send you an update on the progress of these studies.

At this stage, our preliminary study shows only limited degradation of astaxanthin when the algal meal has been stabilized with a natural antioxidant and stored at 20°C for 6 months. Other internal studies have shown that no degradation in astaxanthin occurs during storage for 12 months at -20°C. This should allow for a shelf-life of at least 6 months at room temperature, and 12 months at -20°C.

Thank you very much in advance for the attention that you will give to our notification. If you have any question, do not hesitate contacting us at (808)326-9301.

Sincerely yours,

Martin Guerin
V.P. Marketing & Sales
Aquasearch Inc.

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