
Haematococcus Pluvialis and Astaxanthin Safety For Human Consumption

Safety for human consumption of *Haematococcus pluvialis* algal meal and astaxanthin has been demonstrated by a number of studies:

- A recent 28-day rat study with *Haematococcus pluvialis* dry algal meal, produced by Aquasearch's proprietary technology, demonstrated that there was no observed sub-acute toxicity at a daily dose of 50 mg/kg body weight, corresponding to 3,500 mg algal meal per 70-kg body weight of a typical adult man.
- No lethality was seen for *Haematococcus pluvialis* algae at doses up to 5000 mg/kg body weight, in an earlier, 13-day, single-dose (acute-toxicity), rat study.
- A human safety study demonstrated that daily ingestion of up to 1,140 mg Aquasearch's *Haematococcus pluvialis* algal meal, for 29 days, did not result in any safety concern.
- A recent sub-acute rat toxicity with Aquasearch's *Haematococcus pluvialis* algal meal, showed no signs of toxicity, after dosing rats with up to 1.15 mg astaxanthin per kg body weight per day (equivalent to 80.5 mg astaxanthin per 70-kg body weight) for 28 consecutive days.
- In a human safety study with Aquasearch's algal astaxanthin, no sign of toxicity or safety concern was observed, when volunteers ingested up to 19.25 mg astaxanthin per day for 29 days, while an earlier human study failed to find any harmful effect from 14.4 mg/day astaxanthin ingestion for two weeks.
- Pure astaxanthin (up to 80 mg/kg feed), is Generally Considered As Safe by FDA, for use in salmon diets. This can result in astaxanthin deposition of 10 to 15 mg/kg in salmon fillets. Levels of astaxanthin naturally occurring in wild-caught seafood, and dietary studies on carotenoids, seafood, and salmon, also suggest that a daily serving of 5 mg astaxanthin, corresponding to 125 g of wild-caught Sockeye salmon fillet or less than 100 g of krill, is safe.
- The proprietary technology and quality control developed by Aquasearch to produce *Haematococcus pluvialis* algal meal, ensure that the product meets dietary supplement safety standards.

Conclusion: A supplement containing 5 mg astaxanthin derived from 250 mg, or less, of Aquasearch's *Haematococcus pluvialis* algal meal is safe for daily human consumption.

Aquasearch's proprietary technology allows the production of a high quality algal meal containing 2% total astaxanthin or more. It is therefore a very good source of natural astaxanthin, a carotenoid pigment and biological antioxidant widely encountered in nature. Safety for human consumption of astaxanthin and *Haematococcus pluvialis* algae has been demonstrated by a number of studies.

1. Toxicity studies

1.1. *Haematococcus* algae.

1.1.1. Human safety study

In a recent clinical safety study with Aquasearch's *Haematococcus pluvialis* algal meal, 33 human volunteers (15 males and 18 females, age 28 to 62) ingested on a daily basis, for 29 consecutive days, either a Low Dose supplement containing 228 mg algal meal and 3.85 mg astaxanthin, or a High Dose supplement containing 1140 mg algal meal and 19.25 mg astaxanthin.¹

Volunteers underwent a complete medical examination before, during and at the end of the study. The physician, examined specifically, but not exclusively, the weight, skin coloration, general appearance, blood pressure, vision and eye, (near and distant vision, color vision, depth perception, eye condition), ears and nose, mouth, throat and teeth, chest and lungs, and reflexes, for each volunteer.

This medical examination was complemented by extensive urine analyses and blood analyses (cell counts, hemoglobin, liver enzyme activity indicators, and other blood parameters) (Table 1). No ill effects or toxicity from ingestion of the supplement were observed, confirming the absence of toxicity of Aquasearch's *Haematococcus pluvialis* algal meal.

1.1.2. Rat toxicity studies

Absence of toxicity of *Haematococcus pluvialis* has also been demonstrated in rats and mice, widely accepted animal models for safety assessment of human dietary supplements.

A 28-day sub-acute rat toxicity study, with *Haematococcus pluvialis* algal meal produced with Aquasearch's proprietary technology, failed to find any sign of toxicity of this algal meal.² Three groups of 20 rats each (10 males/10 females) were fed daily by gavage 0, 5, or 50 mg/kg algal meal in a corn oil suspension for 28 consecutive days (corresponding to daily doses of 0, 350 mg and 3,500 mg algal for 70-kg body weight). After sacrifice, the post-mortem observations, hematology and clinical chemistry failed to detect any sign of toxicity.

An earlier 13-day rat toxicity study demonstrated that the LD50 acute toxicity of *Haematococcus pluvialis* algal meal in rats was greater than 5000 mg/kg.³ In this study, three separate groups of 10 rats (5 males and 5 females per group) were fed 5,000 mg/kg algal meal suspended in a 0.5% methylcellulose solution. Mortality, body weights, necropsy examination and pharmacotoxic signs were evaluated on each group. The study found no remarkable differences in body weights or visible abnormalities. The post-mortem examination after sacrificing the animals at the end of the study revealed no abnormalities.

Another acute toxicity trial was reported with male and female mice.⁴ In this study, *Haematococcus pluvialis* algal meal was suspended in distilled water for gavage to give a 30% solution (w/v). The solution was given in a single dose, at dosages ranging from 10,417 to 18,000 mg/kg. No mortalities occurred and no abnormalities were observed in the post-mortem examination. When converted to a 70-kg body weight, these doses are equivalent to single doses ranging from 729 g to 1,260 g.

1.1.3. Other studies

In salmonids, numerous experiments have shown that *Haematococcus pluvialis* can be incorporated in the diet at dosages ranging from 0.1% to 6% without any negative effect on growth or survival.^{5,6,7,8} A recent report showed no indication of disease, toxicity or neoplasia in fish fed *Haematococcus pluvialis* as a dietary source of astaxanthin.⁴ The fish were reported in excellent nutritional status with abundant body fat. Studies have also indicated that feeding *Haematococcus pluvialis* can enhance growth and/or survival in trout and shrimp.⁸⁻¹⁰

1.2. Astaxanthin.

Astaxanthin naturally appears in the human diet when seafood such as salmon, red fishes, shrimp, krill or lobster are eaten.

1.2.1. Human studies

The recent clinical safety study, mentioned above, proved the safety of astaxanthin from Aquasearch's *Haematococcus pluvialis* algal meal.¹ In that study, 33 human volunteers (15 males and 18 females, age 28 to 62) ingested on a daily basis, for 29 consecutive days, either 3.85 mg or 19.25 mg algal astaxanthin. As mentioned earlier, extensive blood and urine analyses were conducted throughout the study (Table 1), and the physician conducted a detailed medical examination. Based on the results of these urine and blood analyses and the observations of the physician, no sign of toxicity from astaxanthin

was detected even at the higher dose.

In a study with healthy human patients, who ingested up to 14.4 mg/day astaxanthin for two weeks, no ill effect was reported.¹¹ On the contrary, a positive antioxidant effect of astaxanthin on serum Low Density Lipoprotein (LDL) was observed. In that study, thirteen healthy patients were selected, subdivided into 3 groups, and given three levels of astaxanthin daily, for two weeks, as follows: 5 patients fed 3.6 mg/day, 5 patients fed 7.2 mg/day, and 3 patients fed 14.4 mg/day. The astaxanthin was administered sublingually in the form of a softgel capsule. Blood samples were taken and the LDL fraction was collected and exposed to an oxidizing agent. The study demonstrated that increasing doses of astaxanthin significantly and increasingly slowed down the oxidation of the LDL fraction.

1.2.2. Rat toxicity studies

In the recent study with Aquasearch's *Haematococcus pluvialis* algal meal, described above, rats ingested daily up to 1.15 mg astaxanthin per kg body weight (equivalent to 80.5 mg for 70-kg body weight per day), for 28 days, without showing any sub-acute toxicity sign.

Other animal studies on the effects of astaxanthin have shown that even higher doses could be fed to rats for prolonged periods. Some of these studies have demonstrated beneficial results. In one study, feeding rats 500 ppm astaxanthin for 34 consecutive weeks resulted in reduced cancer occurrence in the intestinal and oral mucosa and improved the condition of the oral cavity.^{12,13}

1.2.3. Safety of astaxanthin in food salmon – safe daily dose of astaxanthin

For years, astaxanthin has been added to aquaculture diets at levels of up to 200 mg/kg, without any toxic effect on target animals. Additionally, numerous studies have demonstrated improved growth, survival and immune response in fish and shrimp.^{8-10,14-23} Astaxanthin is regularly added at 50 ppm or higher to commercial diets fed to food fish for prolonged periods, i. e., for up to 2 years in the case of farmed salmon.

According to the Code of Federal Regulations, astaxanthin is Generally Recognized As Safe ("GRAS") when used as a color additive in salmon foods, with a maximum inclusion of 80 mg/kg feed.²⁴ Numerous studies have shown that such an inclusion level results in accumulation of astaxanthin in the flesh of Atlantic salmon at levels between 4 and 10 mg/kg, and at even higher levels in other species (Table 2).

These levels in Atlantic salmon are comparable to or slightly higher than levels observed in their wild counterparts, but lower than levels found in other wild salmon species found on the Pacific coast of the United States, where values as high as 58 ppm in Sockeye salmon were reported by a recent FDA study.²⁵ (Average of astaxanthin measurements in this study were 13.8 ppm in Coho salmon and 40.4 ppm in Sockeye salmon).

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*.^{8,25}

Salmon, a fish rich in omega-3 fatty acids, is considered a healthy food, and, like other sources of these poly-unsaturated fatty acids, is highly recommended by nutritionists.²⁶⁻²⁹ According to an epidemiological study on Alaska's native and non-native residents, the lowest rate of ischaemic heart disease mortality, less than one-third that of US Caucasians, occurred in Alaskan Eskimos who lived in an area with documented patterns of high salmon consumption by individuals with high blood concentrations of omega-3 fatty acids.²⁸ Based on the salmon flesh astaxanthin values mentioned above, a daily consumption of a 200-g portion of wild Sockeye salmon with 40 ppm astaxanthin in the flesh would lead to a daily ingestion of 8 mg astaxanthin per day. From a different point of view, the intake of a 5 mg supplement of astaxanthin corresponds to eating 500 g per day of farmed rainbow trout or Atlantic salmon, 125 g of wild Sockeye salmon, or less than 100 g of krill.

Based on these published data, as well as the animal toxicity data publicly available, it may be inferred that the ingestion of 5 mg astaxanthin per day by an adult human is reasonably safe. This was further substantiated by Aquasearch's 29-day human safety study, which investigated the safety of 3.8 mg astaxanthin/day and 19 mg/day astaxanthin from *Haematococcus pluvialis* algal meal, i. e., almost four-fold higher than the assumed safe daily dose of 5 mg.¹

The results of the extensive blood and urine analyses and complete physical examinations before, during, and at the end of the trial period, raised no apparent safety concern. The data were reviewed by two independent physicians, a clinical

pathologist and a professional pharmacotoxicologist, all of who concurred that both doses were safe.

2. Non-mutagenicity of *Haematococcus*

A recent study³⁰ reported no mutagenic effect of *Haematococcus pluvialis* algae, using a mutagenicity test with *Salmonella typhimurium* strains TA100, TA1535, TA98, TA1537, TA1538, and E.coli WP2 uvr A.

In this experiment, *Haematococcus pluvialis* algal meal was formulated in a 50mg/mL solution of dimethyl sulfoxide. The formulation was spread onto petri dishes in the presence of the microbial cultures with positive controls. The positive (mutagenic agents): 2-(2-furyl)-3-5(5-nitro-2-furyl)acrylamide, 1-ethyl-2-nitro-3-nitrosoguanidine, 9-aminoacridine, 2-aminoanthracene, and 2-nitrofluorene, showed a remarkable increase in the number of reverent colonies in every case, compared to the solvent control.

3. Carcinogenicity

Haematococcus pluvialis is not known to have any carcinogenic effect, or contain significant levels of recognized carcinogens. On the contrary, *Haematococcus pluvialis* contains a high level of astaxanthin which has widely demonstrated anticarcinogenic effects.³¹⁻³⁵

4. Heavy metals

Haematococcus pluvialis algae produced and processed by Aquasearch for human food consumption meet the Federal Food and drug Administration's list of maximum tolerances:

- Heavy metals (as lead): < 10.0 ppm
- Mercury < 1.0 ppm
- Cadmium < 0.5 ppm
- Arsenic < 2.0 ppm
- Lead < 5.0 ppm

This has been confirmed by analyses of various batches (Lot HP980051³⁶ and Lot 990610Mix³⁷, a blend resulting from combining five batches: Lots 990513A, 990518B, 990520A, 990524A, 990526A, and therefore, highly representative of the quality of *Haematococcus pluvialis* algal meal produced with Aquasearch's technology).

5. Bacteriology

Manufacturing process follows FDA GMP recommendations for food supplements to avoid spoilage and contamination of *Haematococcus pluvialis* algal meal by harmful micro-organisms or other types of contaminants.

During the processing, the algal biomass is mechanically cell-broken to ensure a thorough rupture of cell walls, undergoes a pasteurization process, and is dried to a moisture content less than 5%. The pasteurization treatment ensures that the following bacteriological specifications in the final product are achieved, as confirmed by analyses by an independent laboratory³⁷:

- Total aerobic plate count <1,000 CFU
- Total coliforms <10/g
- *E. coli* <10/g
- *Salmonella* absence in 25 g

6. Other natural toxic compounds and toxicity risks

Aquasearch is not aware of any significant or detectable levels of known carcinogenic or toxic compounds in *Haematococcus pluvialis* algae that could have a negative effect on human health.

Analyses on the algae meal have demonstrated absence of mycotoxins, and especially of aflatoxins.^{36,37}

Haematococcus pluvialis may contain small amounts of canthaxanthin, a carotenoid pigment closely related to astaxanthin. Analyses have shown that canthaxanthin concentrations in *Haematococcus pluvialis* algal meal produced with Aquasearch proprietary technology are less than 2% of total astaxanthin concentration. Aquasearch's proprietary technology maximizes astaxanthin biosynthesis by *Haematococcus pluvialis* and in so doing also minimizes the relative proportion of other carotenoids (including canthaxanthin).

At the levels of canthaxanthin encountered in Aquasearch's algal meal, a daily dose of 5 mg algal astaxanthin as a supplement would entail also ingesting 0.1 mg canthaxanthin per day. Although canthaxanthin has been demonstrated to have positive metabolic effects such as an anticancer activity,³⁸ there has been reports that, at high doses for prolonged periods, it can have negative effects. One case of aplastic anemia associated with canthaxanthin ingested for tanning purposes, was reported a few years ago³⁹. Others have reported the appearance of crystalline formations in the retina of some individuals who ingested up to 66 g cantaxanthin over 24 months (corresponding to an average daily ingestion of 90 mg cantaxanthin per day) for tanning purposes⁴⁰. However, later it was demonstrated that these canthaxanthin deposits in the retina could be reversed³⁹. In any case, the levels of canthaxanthin that would be ingested through a 5 mg astaxanthin dietary supplement formulated with Aquasearch's algal meal are nearly 1000-fold lower than the doses which were observed to cause canthaxanthin maculopathy. Therefore, they should represent no safety risk.

The rat toxicity and human studies which were conducted with Aquasearch's algal meal confirmed this. It should also be noted that FDA has approved canthaxanthin as a color additive in fish foods (up to 80 mg/kg feed, which can result in canthaxanthin deposition levels of 4 to 12 mg/kg fillet) and broiler diets, as well as in foods and drugs.⁴¹ In foods, the limit authorized by FDA is 30 mg canthaxanthin per pound of solid food. The ingestion of 0.1 mg cantaxanthin in a dietary supplement containing 5 mg astaxanthin, is therefore well below the levels that would be encountered in foods that are considered safe by FDA.

7. Product specifications

A detailed description of the manufacturing process and of the specifications of *Haematococcus pluvialis* for use in dietary supplements are reviewed in a separate technical report.⁴²

8. Metabolic effects of astaxanthin

Astaxanthin is a powerful natural antioxidant. There is a growing amount of scientific evidence not only on the safety of astaxanthin for human consumption, but on the positive metabolic effects that it may have. These findings have been reviewed in detail in Aquasearch Technical Reports TR.3002.001⁴³ and TR.3003.001⁴⁴.

9. Dietary studies - safe daily dose of algal astaxanthin

Astaxanthin appears to be absorbed in the blood in the same way as other carotenoids. Carotenoids are absorbed by passive diffusion through the intestinal mucosa after being emulsified and solubilized in lipid micelles which are incorporated into chylomicrons when exiting the intestinal mucosal cells.⁴⁵ They are transported in the blood after being transferred from the chylomicrons to lipoproteins.

In a recent human study, a single dose of 100 mg dietary astaxanthin was not found to have any negative effect and demonstrated that astaxanthin has a similar absorption pattern to other carotenoids.⁴⁶ Astaxanthin was measured in the blood plasma of 3 middle-aged male subjects after ingestion of a single dose of 100 mg astaxanthin. Astaxanthin was readily absorbed and transported by various lipoproteins: chylomicrons/Very Low Density Lipoproteins, High Density Lipoproteins and Low Density Lipoproteins.

Plasma levels of astaxanthin peaked at 1.2 mg/L (= 2 μ mol/L) after 6 hours and progressively declined over the next 66 hours to a 0.2 mg/L level. These levels and duration are comparable to levels reported in the literature for other carotenoids.⁴⁷⁻⁴⁹ Astaxanthin appears to be absorbed at a similar rate than beta-carotene which peaks in the serum after 6 to 9 h.⁴⁹ In mice, astaxanthin also appeared to be absorbed quite effectively, when compared to beta-carotene or lutein.⁴⁹

The official recommended dietary intake for vitamin A is 1,000 retinol equivalents, for men, and 800 for women.⁵¹ This corresponds to 6 μ g (*micrograms*) beta-carotene or 12 μ g of other pro-vitamin A carotenoids.⁵¹ On the other hand, practical levels of carotenoid intake are significantly higher. Epidemiological studies in North Europe have found daily ingestion of

carotenoids ranging from 2.9 to 7.6 mg/ (*milligrams*) per day,⁵²⁻⁵⁴ while in the US, the level of carotenoids supplied by the "normal" diet is estimated to be 1.5 mg beta-carotene per day.⁵¹

The Alliance for Aging Research, a US Citizen Advocacy organization for research to improve the health and independence of older people, has recommended 10 to 30 mg beta-carotene per day for optimal health, and doses of 20 to 180 mg beta-carotene for many years have been used to treat erythropoietic protoporphyria, with no evidence of toxicity and without development of abnormally-elevated blood vitamin A levels.⁵¹ In addition it should be noted that astaxanthin, unlike other carotenoids such as beta-carotene, has no provitamin A activity;^{55,56} therefore it represents a lower risk of hyper-vitaminosis A.

It may be argued that because astaxanthin is closely related to canthaxanthin it could also have similar toxic effects as those described above. However, the available data indicate that astaxanthin consumption at no greater than the recommended dose of 5 mg per day poses no safety risk:

- The proposed daily intake of astaxanthin (5 mg) is much lower than the levels of canthaxanthin which were found to have toxic effects (up to 90 mg average daily intake for 24 months).
- The human safety study conducted with Aquasearch's algal astaxanthin found no changes in vision or eye condition in the patients. Another good indicator, skin coloration, did not change throughout the Aquasearch safety study.
- The post-mortem examination of the animals in Aquasearch's rat toxicity study also failed to find any adverse effect of astaxanthin supplementation at the doses tested.
- Researchers at the University of Illinois also reported that, in an animal model (rats), astaxanthin, unlike canthaxanthin, did not form crystalline depositions in the eye.⁵⁷ Furthermore, they demonstrated that astaxanthin can have a beneficial role in the protection of the eyes from UV-light damage.

In conclusion, based on published studies (reviewed above), on natural levels of astaxanthin found in seafood, and on the results of the studies conducted by Aquasearch, it appears that the consumption by a healthy adult human of a daily dose of 5 mg astaxanthin, in the form of a supplement formulated with 250 mg (or less) *Haematococcus pluvialis* algal meal produced with Aquasearch's proprietary technology, represents no safety risk. This suggested dose is approximately four times lower than the high dose which was demonstrated to be safe by Aquasearch's safety study.

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Table 1: List of analyses in human safety study conducted on Aquasearch's *Haematococcus pluvialis* algal meal.¹

Blood chemistry analyses

Serum glutamate pyruvate transaminase (SGPT)
 Lactate dehydrogenase (LDH)
 Glucose
 Total protein
 Total bilirubin
 Blood urea nitrogen (BUN)
 Creatinine
 Total cholesterol
 High-density lipoprotein (HDL) cholesterol
 Triglycerides
 Low-density lipoprotein (LDL) cholesterol (calculated)
 Albumin
 Globulin

Complete blood count (CBC)

White blood count (WBC)
 Red blood count (RBC)
 Hemoglobine (HGB)
 Hematocrit (HCT)
 Mean corpuscular volume (MCV)
 Mean corpuscular hemoglobin (MCH)
 Mean corpuscular hemoglobin concentration (MCHC)
 Red cell distribution width (RDW)
 Platelet count
 Neutrophil (segs)
 Lymphocytes
 Monocytes
 Eosinophils
 Bsophils
 Red blood cell morphology
 Coagulation test (activated partial thromboplastin time, PTT)

Urinalysis tests

Color	pH
Appearance	Protein
Specific gravity	Glucose
Leukocyte	Ketones
Nitrite	Urobilinogen
Blood	Bilirubin

Table 2. Levels of of astaxanthin in selected types of seafoods⁸

Astaxanthin			
Seafood type	Content (mg/kg)	Free/esterified	Main isomer
Sockeye salmon	26-37	Free,esterified**	3 <i>S</i> ,3' <i>S</i>
Coho salmon	9-21	Free,esterified**	3 <i>S</i> ,3' <i>S</i>
Chum salmon	3-8	Free,esterified**	3 <i>S</i> ,3' <i>S</i>
Chinook salmon	8-9	Free,esterified**	3 <i>S</i> ,3' <i>S</i>
Pink salmon	4-6	Free,esterified**	3 <i>S</i> ,3' <i>S</i>
Atlantic salmon	3-11	Free,esterified**	3 <i>S</i> ,3' <i>S</i>
Rainbow trout	1-3	Free,esterified**	3 <i>S</i> ,3' <i>S</i>
salmon eggs	0-14	Esterified***	N.A.
Red seabream	2-14	Esterified***	N.A.
Red seabream eggs	3-8	N.A.	N.A.
<i>Peneaus monodon</i>	10-150	Esterified,free**	3 <i>S</i> ,3' <i>S</i>
Lobster		Esterified,free**	N.A.*
Krill	46-130	Esterified***	3 <i>R</i> ,3' <i>R</i>
Krill oil	727	Esterified***	3 <i>R</i> ,3' <i>R</i>
Crayfish meal	137	Esterified***	N.A.*
Artic shrimp	1160	Esterified***	3 <i>S</i> ,3' <i>S</i>
<i>Haematococcus pluvialis</i>	10,000-30,000	Esterified***	3 <i>S</i> ,3' <i>S</i>

* Most crustaceans studied appear to have mostly the 3*S*,3'*S* form, unlike

** depending on tissues, free or esterified astaxanthin may be found

*** also contain a small proportion of free astaxanthin

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