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**STRONG** > Efficiency of Astaxanthin rich algal meal in the treatment of non-ulcer dyspepsia: a randomized double blind placebo controlled study

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Keyword: Dyspepsia, astaxanthin, randomized study

**Background:** No standard therapy for non-ulcer dyspepsia exists. In a pilot study with Hp positive dyspeptic patients astaxanthin rich algal meal effectively reduced intensity of functional heartburn. **Aim:** to evaluate the effectiveness of the carotenoid astaxanthin on dyspeptic symptoms in patients with NUD. **Patients and methods:** Patients referred for upper endoscopy and with established NUD were included. Patients were randomly assigned to receive either 40 mg or 16 mg of astaxanthin rich meal of the green unicellular alga Haematococcus pluvialis (AstaCarox, BioProcess AB, Sweden) or placebo for 4 weeks. Dyspeptic symptoms were assessed with the help of gastrointestinal symptoms rating scale (GSRs); quality of life was evaluated with SF-36 questionnaire at entry, after 4-week treatment and 4 weeks after cessation of medicine. The primary objective of the study was GSRs measured after 4 weeks of treatment. **Results:** 131 NUD patients were included (mean age 43.9± 13.0). 43 patients received 16 mg of astaxanthin, 44 - 40 mg, 44- placebo. Kruskal-Wallis test showed a statistically significant difference between at least two of the treatments groups for the variable Reflux syndrome. Pair-wise comparisons, regarding Reflux syndrome, were performed: Astaxanthin 16 mg vs. Astaxanthin 40 mg, p= 0.0165, Astaxanthin 40 mg vs. placebo p=0.0337, Astaxanthin 16 mg vs. Placebo, p= 0.8409. No other differences in symptoms either QoL were observed. **Conclusion:** Study showed that 4-week treatment with 40 mg of astaxanthin rich algal meal might reduce reflux symptoms in patients with non-ulcer dyspepsia significantly better when compared to 16 mg or placebo.

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## Safety Statement

Herby I undersigned, declare our safety findings in the study titled: "A Phase II randomized, double-blind, placebo controlled, dose response study of the efficacy and safety of Astaxanthin in the treatment of Non-Ulcer-Dyspepsia (NUD) patients with or without *Helicobacter pylori* infection".

The study was randomised, placebo-controlled and double-blinded and included 132 patients.

The code was broken after clean file. The study is not yet published. From the final report the following safety data are found:

### SAE

In total: 1

Causality to Astaxanthin treatment: no relation to the study drug

### AE

In total: 36

Causality to Asta treatment: 11 no relation to study drug  
23 - possible relation  
2 - probable relation

### Plasma concentrations of astaxanthin $\mu\text{g/L}$ :

16-mg/d treatment: ranged from 7 to 31, mean - 20.3

40-mg/d treatment: ranged from 19 to 124, mean - 57,9

Placebo treatment: ranged from 0 to 109, mean - 6,4

High concentrations of astaxanthin in the placebo group (109  $\mu\text{g/L}$ ) and 124  $\mu\text{g/L}$  in the 40 mg treatment group were not related to any AE or SAE.

Limas Kupcinskas MD, PhD.



**ATTACHMENT 7**

**Expert Opinion Report on the Safety of Astaxanthin by Harry G. Preuss, MD, MACN, FAAIM, CNS**



GEORGETOWN UNIVERSITY MEDICAL CENTER

Department of Physiology and Biophysics  
School of Medicine

To Mark Day  
Chief Financial Officer  
Micro Gaia, Inc.

From: Harry G. Preuss MD, MACN, FAAIM, CNS  
Professor of Physiology, Medicine, and Pathology  
Georgetown University Medical Center  
Washington, D.C. 20007

Re: Astaxanthin Safety

Date: 11/20/01, 2001

At your request, I have examined the literature pertaining to the safety of astaxanthin. The report below was derived from the material you forwarded, and material obtained over the internet from PubMed and companies involved in the manufacture of the antioxidant. From the material presented to me by your company, I am also aware that prior astaxanthin notifications to other manufacturers of astaxanthin by the FDA determined that astaxanthin presented essentially no safety issue.

I have divided the report on the safety of astaxanthin into three sections: 1) evidence for safety from dietary findings, 2) animal toxicity studies and 3) human toxicity studies.

#### 1. EVIDENCE FOR SAFETY FROM DIETARY FINDINGS

Astaxanthin is a red pigment that gives color to many living organisms (1). Natural astaxanthin gives salmon and shrimp their red color. Astaxanthin is prevalent in human food stuffs, especially salmon and rainbow trout and also occurs in lobsters and shrimps, in fish eggs, and in other fish species (2). Accordingly, the pigment is found in many human foods and consumed by many. In commercial fish and crustacean farms, astaxanthin is commonly added to feeds to make up for a lack of natural dietary sources (3,4). Indeed, it was found to be necessary for the growth and survival of many organisms (3).

A recent survey of salmonid fish found the following concentrations of astaxanthin ( 5):

SPECIES	ASTAXANTHIN RANGE	ASTAXANTHIN AVERAGE
Wild sockeye salmon	30-58 mg/kg	40.4 mg/kg
Wild Coho salmon	9-28 mg/kg	13.8 mg/kg
Wild pink salmon	3-7 mg/kg	5.4 mg/kg
Wild chum salmon	1-8 mg/kg	5.6 mg/kg
Wild Chinook king salmon	1-22 mg/kg	8.9 mg/kg
Wild Atlantic salmon	5-7 mg/kg	5.3 mg/kg

average of all species = 13.2 mg/kg

Based on these concentrations, the typical fish consumption of 0.25 kg would result in a low intake of 1.325 mg of astaxanthin from the Atlantic salmon to a high intake of 10.1 mg from the sockeye salmon. Based on the all species average concentration, the intake of astaxanthin from salmon would be 3.3 mg. The recommended dose of astaxanthin for nutraceutical use would easily fit in this range.

A ubiquitous algae is the most prevalent commercial source. *Haematococcus pluvalis* algae occurs worldwide and is believed to be the organism which can accumulate the highest levels of astaxanthin in nature. This organism accumulates 1,000 to 3,000 higher levels than salmon. No toxicity associated with *Haematococcus* has ever been reported in the literature (6). The general composition of *Haematococcus* algae consist of common carotenoids, fatty acids, proteins, carbohydrates and minerals. *Haematococcus* algae meal has been approved in Japan as a natural food color and as a pigment in fish feeds.

The structure of astaxanthin is similar to that of other carotenoid pigments like beta carotene; however astaxanthin is a more powerful antioxidant than either beta carotene or vitamin E (7,8). Like other carotenoids, astaxanthin cannot be synthesized in animals and must be provided in the diet. Because of its strong antioxidant capabilities, astaxanthin has been postulated to have some benefit in cardiovascular diseases like atherosclerosis and strokes, macular degeneration, cancer and various neurodegenerative diseases such as Alzheimer's and Parkinson's diseases. Much interest has been focused on the latter two diseases, because astaxanthin can pass the "blood brain barrier", meaning it can deliver antioxidant benefit directly to the brain and central nervous system.

#### SUMMARY

Astaxanthin is a carotenoid and should be as safe as other carotenoids. In addition, astaxanthin has been found safe as an animal supplement and is already in the human food chain.

#### 2. ASTAXANTHIN TOXICITY-RELATED STUDIES IN ANIMALS

Astaxanthin has been approved as feed for salmonid fish for a number of years.

The original petition for use as a feed was filed by Roche Vitamins and is in seven volumes (9). Acute toxicity studies in animals were carried out in rats and mice over a ten day period and doses up to 8000 mg/kg body weight showed no mortality or toxicity. When mutagenicity was tested, no mutations were induced in *Salmonella typhimurium* strain using concentrations of 0.03-0.05 mg/plate in the Ames test whether rat liver homogenates were activated or not. In another test of mutagenicity using the Micronucleus test, no chromosomal breaks nor mitotic dysjunction were found *in vivo* with doses of 500, 1,000, and 2,000 mg/kg of body weight. Concerning teratology and embryotoxicity studies, doses of 100, 200, and 400 mg/kg/day were given to pregnant rabbits for a 12 day period. At 30 days of gestation, the examination of fetuses showed no malformations. The mothers all tolerated the astaxanthin well. In rats given astaxanthin, no embryotoxic or teratogenic effects of astaxanthin were observed. Reproductive studies on rats and dogs revealed no perturbations from the high doses used. Thirteen week tolerance tests on rats and dogs revealed no toxic effects.

Three different lots of *Haematococcus* algae were administered by gavage to Charles River CD rats (9). 5,000 mg/kg were administered in a 0.5% aqueous methylcellulose solution. In a thirteen day study on both male and female rats, no visible abnormalities were noted and the rats continued to gain weight during the entire study. In another study, doses of 10,417-18,000 mg/Kg given by oral gavage did not reveal any toxicity, even when organs were examined postmortem. Therefore, the LD 50 was judged to exceed 18,000 mg/kg.

A 13-week oral, repeated-dose toxicity study of *haematococcus color*, a food additive mainly composed of astaxanthin, was conducted in male and female F344 rats (10). Rats were randomly divided into four groups each consisting of 10 males and 10 females and given a powder diet containing 0, 0.025, 0.075, and 0.25% *haematococcus*, i.e., 0, 0.5, 1.5, and 5% as the product. None of the animals died during the administration period. There were no exposure-related changes in body weight gain or food consumption. Serum biochemical examinations showed a dose-related increase in cholesterol, but the differences were slight and not defined as an adverse effect. No effects related to treatment were noted in the hematological examinations and organ weights, and no abnormalities that could be ascribed to exposure to *haematococcus color* were observed in histopathological examinations. The authors concluded that ingestion of *haematococcus color* in the diet for 13 weeks did not cause any toxicological changes in F344 rats.

Three groups of male Wistar rats (130-140g) were fed 30 days with synthetic diets containing 0.1% of beta carotene, canthaxanthin, and astaxanthin (11). Another group was fed with a synthetic diet without carotenoids. The results showed that the beta carotene did not induce change in plasma cholesterol, but canthaxanthin and astaxanthin induced a significant increase in cholesterol concentration. However, the increase was due mainly to HDL, the good cholesterol.

Fish tissues from a Haematococcus algae feeding to rainbow trout were analyzed for toxic effects and neoplasia (1). All tissues examined were normal in appearance with no indication of disease, toxicity, or neoplasia. All fish examined were in excellent nutritional status with abundant body fat. Gross findings indicate that no adverse effects on health were observed from Haematococcus algae meal as the dietary source of astaxanthin.

#### **SUMMARY**

Animal studies, where extremely high doses of astaxanthin were used, show a wide range of safety in the use of this antioxidant.

### **3. ASTAXANTHIN TOXICITY-RELATED STUDIES IN HUMANS**

Astaxanthin was administered daily over two weeks to five subjects at 3.6 mg/day, to five subjects at 7.2 mg/day and to three subjects at 14.4 mg/day. A physiological effects on oxidized LDL was noted, but no adverse effects were reported at any dose (12).

Lignell (13) followed 40 healthy volunteers over six months: one half received one capsule containing four mg astaxanthin (algae meal) and the other one half received a placebo. No significant differences were observed between the two groups with the exception of the strength endurance tests. These improved on the antioxidant.

Thirty-three healthy adult volunteers were given astaxanthin obtained from Haematococcus pluvialis dry algae meal. Each subject consumed daily over 29 days either a low dose (3.85 mg) or a high dose (19.25 mg) astaxanthin. No adverse reactions were found after examining weight, skin coloration, general appearance, blood pressure, near and distant vision, color vision, depth perception, general eye condition, ears and nose, mouth, throat and teeth, chest and lungs, reflexes, blood analyses and urinalyses.

#### **SUMMARY**

Although the studies are limited, human trial to date show no adverse events from consumption of the nutraceutical.

### **CONCLUSIONS**

In light of this information, there appears to be no question as to the safety of Micro Gaia's astaxanthin product. In addition, I believe that Micro Gaia and the Federal Food and Drug Administration can reasonably rely upon the Agency's prior acceptance of safety data submitted by other companies marketing astaxanthin as a "new dietary ingredient" in the United States. All evidence points to complete safety in the use of astaxanthin. Astaxanthin deserves no more concern when used in proper dosing than the use of other carotenoids such as beta carotene.

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