

## Astaxanthin

The antioxidant benefits of the carotenoid Astaxanthin have only recently been fully understood, and human clinical trials are still in the early stages. However the known, as well as the potential benefits of this red pigment, found in fish and crustaceans, are extensive.

Astaxanthin is fat-soluble, pigment antioxidant called a xanthophyll belonging to the carotenoid family of compounds. There are over 600 carotenoids found in nature providing the red, orange and yellow pigments to fruits and vegetables. The best-known carotenoid is probably beta-carotene, the pro-vitamin A found in carrots and other orange vegetables. Beta-carotene is converted to vitamin A by the human digestive system. The carotenoids are broadly divided into "carotenes," or non-oxygen substituted hydrocarbon carotenoids, and "xanthophylls," oxygen-substituted carotenoids. Carotenoids are chemically known as tetra-terpenes, a subgroup of the large group of plant constituents known as terpenes or terpenoids. Carotenoids are large molecules with a backbone row of forty Carbon molecules (a hydrocarbon chain with conjugated double bonds). Lycopene and lutein are other carotenoids, which have become very popular in the last few years because of their beneficial effects on the prostate and vision respectively. Lycopene, lutein and many other carotenoids lack pro-vitamin A like activity.[1]<sup>1</sup>

Astaxanthin is found in birds and fish. Most crustaceans, including shrimp, crawfish, crabs and lobster, are tinted red by accumulation of astaxanthin. The pink flesh of wild salmon is also due to astaxanthin. In aquaculture, where there is a lack of a natural dietary source of the pigment, astaxanthin is used to maintain the pink color desired by consumers. Astaxanthin has been found not only to provide the pigmentation but also to be essential for the proper growth and health of the fish.[2,3]<sup>2</sup>, <sup>3</sup> Astaxanthin is also used in the poultry industry to enhance the color of the egg yolk. Astaxanthin is also found in certain plants and algae. In fact, animals cannot synthesize astaxanthin and the algae and plants provide the source of the carotenoid.[4]<sup>4</sup>

Astaxanthin is commercially produced from the freshwater algae, *Haematococcus pluvialis*. However, natural astaxanthin may also be produced from the pink yeast *Xanthophyllomyces dendrorhous*, from the yeast *Phaffia rhodozyma* or it can be extracted from the by-products of crustacean such as the Antarctic krill. The commercial aquaculture industry use synthetically produced astaxanthin in fish farms.[5]<sup>5</sup>

*Haematococcus pluvialis* is a freshwater alga of the Chlamydomonadeae family. Under unfavourable growing conditions, such as in nutrient deficient and dry environment, the algae lose their motility and form spores with thick cell walls. During the transformation into this sporal phase, the algae accumulate starch and fats in their cells for energy and carbon stores. During the production of fats, astaxanthin is synthesized to prevent lipid peroxidation and to protect the DNA in the cell nucleus from UV radiation. This process is exploited commercially with the algae growing under hygienic conditions in tanks or ponds. After maximizing growth the environment is then challenged, mimicking the environmental stresses that in nature stimulate the algae to biosynthesize astaxanthin and the green alga turns red. The cell walls of the algae are broken to improve the bioavailability of astaxanthin. The algal biomass is then pasteurized and gently dried at temperatures below 100° C to minimize loss of nutrients, especially astaxanthin. The end result is a dark red powder

rich in astaxanthin and other carotenoids. The powder can subsequently be purified as needed.[6,7]<sup>6, 7</sup> Astaxanthin can also be produced synthetically. Synthetic astaxanthin is produced by Sigma, Hoffmann-La Roche AG and BASF AG. As well as pure Astaxanthin, there are also various derivatives including Cardex<sup>TM</sup> (disodium disuccinate astaxanthin).[8]<sup>8</sup>

### **Synthetic versus natural astaxanthin**

The atoms comprising an astaxanthin molecule can be oriented in different ways, producing different isomers. The most common geometric configuration in both synthetic and natural astaxanthin is the more thermodynamically stable all-*E* (all-*trans*) isomer. Astaxanthin from natural sources tends to occur predominantly as either the (3*S*,3'*S*) or (3*R*,3'*R*) form, while the (3*R*,3'*S*) isomer is the most abundant in synthetic astaxanthin.[9]<sup>9</sup> The significance of this in humans is not known but natural astaxanthin tends to produce higher pigmentation in rainbow trout compared to synthetic astaxanthin provided at the same dietary concentration.[10]<sup>10</sup>

*Table 1: Distribution of astaxanthin stereoisomers[11]<sup>11</sup>*

Source	Isomers		
	(3 <i>S</i> ,3' <i>S</i> )	(3 <i>R</i> ,3' <i>R</i> ) and (3 <i>R</i> ,3' <i>S</i> )	(3 <i>R</i> ,3' <i>R</i> )
<i>Phaffia spp.</i> (yeast)	-	<2%	>98%
<i>Haematococcus pluvialis</i> (algae)	100%	-	-
Synthetic astaxanthin	25%	50%	25%
Atlantic salmon	78-85%	2-6%	12-17%

### **Pharmacological studies**

#### **Antioxidant activity**

The carotenoids are excellent singlet oxygen quenchers as well as lipid peroxidation chain-breakers; this dual antioxidant capacity is generally attributed to the activity of the polyene chain, and increases with the number of conjugated double bonds along the polyene chain length.[12]<sup>12</sup> Because of its chemical structure, astaxanthin is able to sit inside the phospholipid structure of the cell membrane.[36]<sup>36</sup> Astaxanthin has been shown to exert superior antioxidant properties to beta-carotene in a number of in vitro studies.[13-16]<sup>13, 14, 15, 16</sup> The antioxidant activity of astaxanthin has been found to be 80 times stronger than alpha-tocopherol and twice as strong as betacarotene.[17,36]<sup>17, 36</sup>

The carotenoid significantly increased the activities of the antioxidant enzymes catalase (CAT) and superoxide dismutase (SOD), the effect was superior to that of beta-carotene.<sup>20</sup> The antioxidant activity has also been confirmed in the recently developed fluorometric assay. Astaxanthin was shown to be a more potent antioxidant than alpha-tocopherol, alpha-carotene, lutein, beta-carotene and lycopene in organic and liposomal media. 18UV radiation from sunlight is the most potent environmental risk factor in skin cancer pathogenesis. Astaxanthin has been shown to protect against UVA light-induce oxidative stress in vitro.<sup>19, 20</sup>

Astaxanthin has also been found to be liver protective. The carotenoid was shown to protect liver against carbontetrachloride by inhibiting lipid peroxidation and stimulating the cellular antioxidant system in rats. Astaxanthin blocked the increase of glutamate-oxalacetate transaminase (GOT) and glutamate-pyruvate transaminase (GTP) activity and thiobarbituric acid reactive substances (TBARS) in response to carbon tetrachloride, while causing an increase in glutathione (GSH) levels and superoxide dismutase (SOD) activities.<sup>21</sup> Antioxidants may also help reduce muscle injury from strenuous exercise. Astaxanthin has been shown to attenuate exercise-induced damage in mouse skeletal muscle and heart, including an associated neutrophil infiltration that induces further damage.<sup>22</sup>

### **Gastric Ulcer protective**

*Helicobacter pylori* is a gram-negative bacterium affecting about half of the world population, causing chronic gastritis. In some patients the disease evolves into gastric ulcer, duodenal ulcer and gastric cancer. The pathogenesis is partly immunological. The mucosa responds by activating phagocytes and T-lymphocytes, which produce IFN-gamma leading to increased mucosal inflammation and damage. A low dietary intake of antioxidants such as carotenoids and vitamin C may be an important factor for acquisition of *H. pylori* and supplementation may be beneficial.<sup>23, 24</sup> Treatment of *H. pylori* infected mice with an algal cell extract containing astaxanthin has been shown to reduce the bacterial load and gastric inflammation in mice. The anti-inflammatory effect of astaxanthin is associated with a shift of the T-lymphocyte response from a predominant Th1-response dominated by IFN-gamma to a Th1/Th2-response with IFN-gamma and IL-4.<sup>23</sup> Astaxanthin may also have a role in preventing drugs, such as the non-steroidal anti-inflammatory drug, Naproxen, from inducing gastritis and ulceration. The oral administration of astaxanthin (1, 5, and 25 mg/kg of body weight) has been shown to significantly protect against naproxen (80 mg/kg of body weight)-induced gastric antral ulcer and inhibit elevation of the lipid peroxide level in gastric mucosa in rats. In addition, pretreatment of astaxanthin resulted in a significant increase in the activities of radical scavenging enzymes such as superoxide dismutase, catalase, and glutathione peroxidase. A histologic examination clearly proved that the acute gastric mucosal lesion induced by naproxen nearly disappeared after the pretreatment of astaxanthin. These results suggest that astaxanthin removes the lipid peroxides and free radicals induced by naproxen.<sup>25</sup>

### **Cardiovascular activity**

Observational and epidemiological studies suggest that carotenoids have cardioprotection activities in humans. A derivative of astaxanthin (disodium disuccinate Astaxanthin (Cardax) ) has been found to act as a myocardial salvage agent in an animal model of infarction. Cardax at 50 and 75 mg/kg i.v. for 4 days resulted in a significant mean reduction in the size of the infarction and area of risk. The infarct size and myocardial salvage were significantly, and linearly, correlated with plasma levels of non-esterified, free astaxanthin at the end of reperfusion. These results suggest that parenteral astaxanthin/Cardax may be a beneficial in reducing the risk of cardiac infarction.<sup>26</sup> The cardioprotective activities may also be related to anticommplement activity.<sup>27</sup>

The composition of atherosclerotic plaques, not only macroscopical lesion size, has been implicated in their susceptibility to rupture and increase the risk of thrombus formation. Alpha-tocopherol and especially astaxanthin, although they did not affect

lipid accumulation, have been shown to significantly decreased macrophage infiltration in the plaques in hyperlipidaemic rabbits. Thirty-one rabbits were divided into three groups and were fed a standard diet, as controls (N =10), or a standard diet with the addition of 500 mg alpha-tocopherol per kg feed (N =11) or 100 mg astaxanthin per kg feed (N =10) for 24 weeks. Astaxanthin and alpha-tocopherol was shown to improve plaque stability possibly by decreasing macrophage infiltration and apoptosis in the atherosclerotic plaque. It is suggested that apoptosis reduction by alpha-tocopherol and astaxanthin may be a new anti-atherogenic property of these antioxidants.<sup>28</sup> Another study found that astaxanthin inhibits low-density lipoprotein (LDL) oxidation and the prevention of atherosclerosis may therefore also be due to the beneficial effects on this lipoprotein.<sup>29</sup>

However in another study, alpha-tocopherol, but not astaxanthin, was shown to have a beneficial effect on LDL oxidation. Thirty-one, 3-month-old heritable hyperlipidemic rabbits were divided into three experimental groups. One group (n=10) was fed standard rabbit feed alone and served as a control, a second group (n=11) was supplied with the same feed containing 500 mg alpha-tocopherol/kg and a third group (n=10) was given a feed containing 100 mg astaxanthin/kg. Plasma lipids, lipoproteins and LDL oxidation lag time were followed for 24 weeks. Neither compounds prevented atherogenesis in this study of heritable hyperlipidemic rabbits.<sup>30</sup>

Astaxanthin has also been shown to lower the blood pressure in spontaneously hypertensive rats. The antihypertensive effect was not observed in normtensive animals. The long-term administration of astaxanthin (50 mg/kg) for 5 weeks in stroke prone rats induced a significant reduction in the blood pressure; it also delayed the incidence of stroke. The carotenoid was also found to have a significant neuroprotective effects in ischemic mice, presumably due to its antioxidant potential. Pretreatment of the mice with astaxanthin significantly shortened the latency of escaping onto the platform in the Morris water maze learning performance test. In conclusion, these results indicate that astaxanthin can exert beneficial effects in protection against hypertension and stroke and in improving memory in vascular dementia.<sup>31</sup> The antihypertensive effect of astaxanthin may be due to a NO-related mechanism and by modulating the blood fluidity. Astaxanthin may normalize the sensitivity of the adrenoceptor sympathetic pathway, particularly [alpha]-adrenoceptors, and by restoring the vascular tone through attenuation of vasoconstriction induced by angiotension II or reactive oxygen species.<sup>32</sup>

## **Immune enhancing and anti-inflammatory effects**

Early studies demonstrating the ability of dietary carotenes to prevent infections, led researchers to suggest that the effects of these carotenoids were due to their conversion to vitamin A. Subsequent studies, however, found that dietary carotenoids without provitamin A activity such as lutein, canthaxanthin, lycopene and astaxanthin also had beneficial effects. In fact, these nonprovitamin A carotenoids were as active, and at times more active, than beta-carotene in enhancing cell-mediated and humoral immune response in animals and humans.<sup>33</sup> Astaxanthin has been shown to enhance antibody production in vitro.<sup>34</sup> Astaxanthin has also been shown to augment immunoglobulin production by peripheral blood mononuclear cells obtained from adult volunteers and full-term newborn babies (umbilical cord blood) in response to various T-cell dependent antigens.<sup>35</sup> Astaxanthin is also a potent anti-inflammatory agent. A recent study found that astaxanthin suppressed the development of

endotoxin-induced uveitis in rats in a dose-dependent fashion. The anti-inflammatory effect of 100 mg/kg astaxanthin was as strong as that of 10 mg/kg prednisolone. Astaxanthin also decreased production of nitric oxide, activity of inducible synthase (NOS), and production of prostaglandin E2 and TNF-alpha in mouse macrophage cell line in vitro in a dose-dependent manner.<sup>36</sup> The anti-inflammatory effect of astaxanthin, is thought to be mediated by the blocking of NF-kappaB activation leading to a reduction in the production of inflammatory mediators.<sup>37</sup> The anti-inflammatory activity of astaxanthin may also have a role in the prevention or treatment of asthma. Ginkgolide from *Ginkgo biloba*, astaxanthin or their combination has been shown to suppress T-cell activation. The effect was as good or better than the commonly used antihistamines cetirizine dihydrochloride and azelastine.<sup>38</sup>

## Anticancer

Chemoprevention, by definition, is the use of one or several chemical compounds to prevent, stop or reverse the development of cancer. It is an attempt to use natural and synthetic compounds to intervene in the early stages of cancer, before invasive disease begins. Chemopreventive agents can act in two ways: they can prevent or stop the genetic mutations that lead to cancer, or they can prevent or stop the processes that promote proliferation.<sup>39</sup> Various natural carotenoids have been shown to have chemopreventive activity, and some of them have been demonstrated to be more potent than beta-carotene. The carotenoids including alpha-carotene, lutein, zeaxanthin, lycopene, beta-cryptoxanthin, fucoxanthin, astaxanthin, capsanthin, crocetin and phytoene, as well as beta-carotene, may be useful for cancer prevention.<sup>40</sup> Carotenoids have also been shown to inhibit the proliferation of human breast cancer MCF-7 cell line in vitro<sup>41</sup> and oral administration (50 p.p.m) of astaxanthin has been shown to reduce the incidence of pre-neoplastic lesions and neoplasm in mice given a bladder carcinogen. The carotenoids significantly reduced the incidence of bladder cancer (transitional cell carcinoma) ( $P < 0.003$ ). These results indicate that astaxanthin is a possible chemopreventive agent for bladder carcinogenesis and that the effect may be partly be due to suppression of cell proliferation.<sup>42</sup> Astaxanthin has also been shown to reduce the occurrence and development, of chemically induced oral cancer in rats<sup>43</sup> and inhibit the growth of mammary tumors in mice.<sup>44</sup> Mammary tumor growth inhibition by astaxanthin was dose-dependent and was higher than that of canthaxanthin and beta-carotene. Lipid peroxidation activity in tumors was lower ( $P < 0.05$ ) in mice fed 0.4% astaxanthin, but not in those fed beta-carotene and canthaxanthin. One of the mechanisms by which astaxanthin exerts antitumor activity may be via enhancement of immune responses. A study in mice found that astaxanthin suppressed transplanted fibrosarcoma tumor cell growth and stimulated immunity against tumor antigen. The astaxanthin-fed mice (0.02%, 40 micrograms/kg body wt/day) had significantly lower tumor size and weight than controls when supplementation was started one and three weeks before tumor inoculation.<sup>45</sup> Astaxanthin also improves antitumor immune responses by inhibiting lipid peroxidation induced by stress. Mice subjected to restraint stress had reduced total number of spleen cells and reduced NK cell activity. The stress also caused a significant increase in the lipid peroxidation of liver tissue. Astaxanthin (100 mg/kg/day, p.o., 4 days) improved the immunological dysfunction induced by restraint stress. The restraint stress also promoted hepatic tumours induced by inoculation. Daily oral administration of astaxanthin (1 mg/kg/day, p.o., 14 days)

markedly attenuated the promotion of hepatic metastasis. These results suggested that astaxanthin improves antitumor immune responses by inhibiting of lipid peroxidation induced by stress.<sup>46</sup> A recent review suggests that the antineoplastic properties appear tightly correlated to their ability to induce the gap junctional protein connexin 43 (Cx43). Upregulation of Cx43 leads to decreased proliferation and decreased indices of neoplasia in animal and human cells.<sup>47</sup>

### **Antidiabetic activities**

Oxidative stress induced by hyperglycemia is one of the causes of pancreatic beta-cell dysfunction and other tissue damage in patients with diabetes mellitus. Astaxanthin was shown to reduce the progressive destruction of pancreatic beta-cells in an animal model of type 2 diabetes. Diabetic obese mice were treated with astaxanthin starting a 6 weeks at age and evaluated at 10, 14 and 18 weeks by non-fasting blood glucose levels, intraperitoneal glucose tolerance test including insulin secretion, and beta-cell histology. Astaxanthin significantly reduced the blood glucose level in the diabetic mice compared to controls. The ability of islet cells to secrete insulin, as determined by the intraperitoneal glucose tolerance test, was preserved in the astaxanthin-treated group. Histology of the pancreas revealed no significant differences in the beta-cell mass between astaxanthin-treated and -untreated diabetic mice. These results suggest that astaxanthin may exert beneficial effects in diabetes by decreasing glucose toxicity and preserving beta-cell function.<sup>48</sup> Astaxanthin has also been found to reduce diabetic nephropathy induced by oxidative stress in mice. The carotenoid reduced oxidative stress on the kidneys and prevented renal cell damage in a rodent model of type 2 diabetes.<sup>49</sup>

### **Human Studies**

There are only few human studies with astaxanthin and none of them have been published in peer-reviewed journals. The following studies have all been sponsored by companies manufacturing or promoting astaxanthin-containing products. More, well-designed clinical trials are needed to confirm the proposed benefits of astaxanthin.

Astaxanthin has been demonstrated to stimulate the immune response and decrease DNA damage in healthy subjects. Free-living healthy female subjects (average age 21.5 yr) with no history of diabetes, cancer, alcohol abuse, or smoking received 0, 2, or 8 mg astaxanthin (n = 14) daily for 8 wk in a double-blind, placebo controlled study. Astaxanthin was found to stimulate concanavalin A-, phytohemagglutinin- and pokeweed mitogen-induced lymphoproliferation and increase NK cell cytotoxic activity. In addition, astaxanthin also increased the proportion of total T cells and B cells, but did not influence the populations of Th, Tc or NK cells or the ratio of Th:Tc cells. Lymphocyte function-associated antigen 1 (LFA-1) is a membrane glycoprotein involved in a wide variety of functional activities mediated by human leukocytes. Expression of LFA-1 is required on immunoregulatory lymphocytes for functional activity. The frequency of cells expressing LFA-1 marker was higher in subjects given 2 mg (42.1%) but not those given 8 mg (30.6%) astaxanthin compare to control (31.8%) on week 8. Delayed type hypersensitivity (DTH) reactions are antigen-specific, cell-mediated immune responses, which, depending on the antigen involved; mediate beneficial (resistance to viruses, bacteria, fungi, and tumors), or harmful (allergic dermatitis, autoimmunity) aspects of immune function. Subjects fed 2 mg but

not those fed 8 mg astaxanthin had higher DTH response than un-supplemented controls. Dietary astaxanthin dramatically decreased blood DNA damage (8-oxodeoxyguanosine) after 4 weeks of feeding but did not influence lipid peroxidation in plasma.<sup>50</sup>

A Japanese patent application reports that a positive antioxidant effect of astaxanthin on serum low-density lipoprotein (LDL) has been observed. Thirteen healthy patients were divided 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.<sup>66</sup>

Cyanotech Corporation reports that their product, Bioastin, has been shown in double blind, placebo-controlled studies to be beneficial in rheumatoid arthritis (RA) and carpal tunnel syndrome (CTS). The clinical trials were conducted by Dr. Gene Spiller at the Health Research Studies Center in Los Altos, California, and were double-blinded, placebo-controlled studies. The RA clinical trial involved 21 patients with a group of 14 randomized patients consuming three BioAstin soft gel capsules daily with each meal for eight weeks and seven randomized patients consuming a placebo. The CTS clinical trial involved 20 patients with a group of 13 randomized patients consuming three BioAstin soft gel capsules daily with each meal for eight weeks and seven randomized patients consuming a placebo. In the RA study, subjects completed a health assessment questionnaire at the beginning of the study and after four and eight weeks. In the CTS study, subjects completed a Symptom Severity Survey at the same time frames.

The RA clinical trial showed a statistically significant improvement in symptoms of RA in patients who consumed BioAstin compared to the placebo group. The CTS clinical trial showed a trend toward decreased wrist pain for patients consuming BioAstin compared to the placebo group. The RA study concluded that BioAstin may be an important addition to RA treatment allowing patients to have less pain and more satisfaction with their ability to perform their usual activities. The CTS study concluded that BioAstin may be an effective treatment for CTS allowing patients to suffer less daytime pain with a shorter duration of pain. The clinical trials were followed by open label studies with volunteers from each trial. Preliminary results from the open label study are said to be very positive.<sup>51</sup> Another study sponsored by Cyanotech reports that BioAstin has been demonstrated to delay the onset of muscle soreness after exercise. Nine weight trained males participated in this study. The subjects ingested either 8 g daily of astaxanthin (n = 4) or a placebo (n = 5) for a 3-week loading phase prior to the muscle soreness-inducing protocol, and during a 12-day recovery phase. Perceptions of delayed onset muscle soreness at 48 hrs post-eccentric exercise were quantified by muscle soreness ratings (0–7 Likert scale). Muscle fiber characteristics were determined via mATPase histochemistry and digital imaging to determine percentage cross-sectional areas of the major fiber types (I, IIA, IIAB/B). Due to small numbers of IIB fibers in some subjects, IIAB hybrid fibers were included in this fiber type population. No differences in perceptions of soreness between the astaxanthin and the control groups were observed ( $p > 0.05$ ), with all subjects exhibiting a mean score of  $> 5$ . Percent fiber type areas were similar ( $p > 0.05$ ) for both groups. However, 48 hrs after the muscle soreness-inducing session, perceptions of soreness did reduce in men with a high percentage area for fiber types

IIA & AB/B.<sup>52</sup> Cyanotec also reports that BioAstin containing 4 mg astaxanthin for two weeks reduced the level of sunburn in 21 subjects.

Astaxin, the product manufactured by the Swedish company, AstaReal, has been the subject of a number of clinical studies. Supplementation with Astaxin was shown in a study to significantly improve muscle strength and endurance in 40 young men.<sup>53</sup> An open clinical study has shown that Astaxin strongly alleviated symptoms associated with dyspepsia including stomach pain and dyspepsia. Ten *Helicobacter pylori* infected patients improved on 4 mg/day of Astaxanin. Seven of the patients reported a reduction in symptoms. In six of the patients, there was a reduction in the level of inflammation in the gastric lining while one patient was fully cured of *H. pylori* infection. In a study of patients operating visual display terminals found that 5 mg/day Astaxin for 4 weeks reduced the subjective level of eye strain by 46%. In another double blind study, 6 mg/day astaxanthin improved depth perception and critical flicker fusion in 20 year old men suggesting that astaxanthin may improve visual acuity.<sup>54</sup>

Clinical observations suggest that a combination of zinc and folic acid, or astaxanthin (AstacaroX), or an energy-providing combination containing (actyl)-carnitine (Proxeed) may improve the quality and function of the sperm in infertile men.<sup>55</sup>

## **Safety**

The LD<sub>50</sub> acute toxicity of *Haematococcus pluvialis* algal meal in rats is greater than 5000 mg/kg. The post-mortem examination after sacrificing the animals at the end of the study revealed no abnormalities.<sup>56</sup> A 28-day sub-acute rat toxicity study failed to find any sign of toxicity from up to 5-50 mg/kg algal meal. After sacrifice, the post-mortem observations, hematology and clinical chemistry failed to detect any sign of toxicity.<sup>57</sup> A study in mice has also failed to detect any abnormalities from a single dose of 10 to 18 g/kg body weight.<sup>58</sup> Astaxanthin is routinely used in aquaculture without any toxic effects on fish and prawns. In fact, astaxanthin, has been shown to benefit the immunity, growth, eye health and survival of the stock.<sup>59, 60, 61, 62</sup>

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. This level of feeding 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.<sup>63</sup>

Astaxanthin has been shown not to be mutagenic in a test using various strains of *Salmonella typhimurium*.<sup>64</sup> *Haematococcus pluvialis* is not known to have any carcinogenic effect, or contain significant levels of recognized carcinogens. On the contrary the algae contains a high level of astaxanthin, which has been shown to have anticarcinogenic effects. In a recent clinical safety study with Mera Pharmaceuticals's *Haematococcus pluvialis* algal meal (AstaFactor), 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. 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). No ill

effects or toxicity from ingestion of the supplement were observed, confirming the absence of toxicity of *Haematococcus pluvialis* algal meal.<sup>65</sup> A Japanese patent application reports that no ill effect were observed in a study with healthy human patients, who ingested up to 14.4 mg/day astaxanthin for two weeks.<sup>66</sup> Thirty-five healthy adults age 35-69 years enrolled in a randomized, double-blind, placebo-controlled trial of 8 weeks' duration consumed three astaxanthin capsules per day, one at each meal. Nineteen participants received capsules with an algal extract in safflower oil, containing 2 mg of astaxanthin each (treatment); 16 participants received soft gel capsules 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 CO<sub>2</sub> 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.<sup>67</sup>

## **Possible Benefits of Astaxanthin**

### **Ageing, sun damage and cancer**

Combining astaxanthin with other fat-soluble and water-soluble antioxidants supplying a wide spectrum of antioxidants is likely to most beneficial in reducing degenerative disease, cancer and ageing. Astaxanthin be particularly effective against UVB-induced damage. It is being promoted as an “internal sunscreen”. However, the initial damage from the sun is to the skin surface. UVA does get to the dermal layers but UVB radiation does not penetrate very far. Both types are responsible for damage to the body's immune system that can lead to skin cancers. Antioxidants are useful for preventing cellular damage, but the surface still needs to be protected.

### **Cardiovascular disease**

Astaxanthin inhibits low-density lipoprotein oxidation, preventing atherosclerosis, stabilizing atherosclerotic plaque, reducing the risk of myocardial infarction and lowering elevated blood pressure, suggesting that it may be useful for reducing the risk of cardiovascular disease.

### **Neurodegenerative disease**

The antioxidant, anti-inflammatory and neuroprotective activities, combined with its ability to cross the blood-brain barrier,<sup>11</sup> suggests that astaxanthin may be beneficial in the prevention and treatment of neurodegenerative diseases including Alzheimer's disease and ocular diseases.

### **Liver disease**

The hepatoprotective activity of astaxanthin may protect the liver against chemicals and lipid peroxidation.

## **Exercise recovery**

Astaxanthin has been shown to attenuate exercise-induced damage in skeletal muscle including an associated neutrophil infiltration that induces further damage.

## **Gastritis and ulceration**

Astaxanthin may be beneficial in the prevention and treatment of *Helicobacter pylori* infection, gastritis and ulceration.

## **Diabetes**

In vitro and in vivo studies suggest that astaxanthin may be beneficial in the prevention of diabetic complications including retinal and renal neuropathies.

## **Inflammation**

Astaxanthin may possibly have a beneficial role in the reduction of inflammation in a variety of chronic inflammatory disease including arthritis, carpal tunnel syndrome and autoimmune diseases.

## **Dosage**

Wild salmon primarily obtain astaxanthin from ingesting krill. Farmed fish obtain it from their diets artificially enriched with synthetic astaxanthin or algae meal. A meal consisting of a 6 oz Atlantic salmon steak would typically contain between 1 and 2 mg astaxanthin. Alaskan Sockeye salmon may contain twice as much astaxanthin.<sup>11</sup> Astaxanthin as a supplement is available in three forms; Algae meal, which is the dried alga in its whole form, oleoresin-rich carbon dioxide (CO<sub>2</sub>) extracts and synthetic astaxanthin. The algae meal typically contains 2% total astaxanthin mainly as monoesters, but also some di-esters and free astaxanthin. CO<sub>2</sub> is widely used in the food industry to extract, among other compounds caffeine from coffee beans and flavour from hops. Typically 10 kg of the dried algae is used to produce 3 kg of the oleoresin rich CO<sub>2</sub> extract, which contains about 10% total carotenoids containing about 80% astaxanthin with the other 90% consisting of mainly lipids and water.<sup>11</sup> Based on published data the ingestion of 6 mg astaxanthin per day by an adult human can be considered safe.<sup>67</sup>

### **Algae Meal**

- The recommended dosage of algae powder meal is about 250 mg; containing 5 mg astaxanthin daily.

### **Extracts of *Phaffia rhodozyma* (yeast) or *Haematococcus pluvialis* (algae)**

- Extracts equivalent to 5 mg astaxanthin daily.

### **Standardised Oleoresin Extracts**

- The recommended dosage for CO<sub>2</sub> extracts is 50-70mg per day (depending on the concentration of the extract) standardized to provide 5 mg astaxanthin daily.

It is recommended that astaxanthin be taken together with a meal containing fats to improve the absorption. Astaxanthin may be mixed with a carrier vegetable or fish oil in a soft gel capsule for the same reasons.

### **Bioavailability**

Delivery of natural carotenoids can be compromised by poor bioavailability. To overcome this, a synthetic water-dispersible derivative of astaxanthin has been synthesized and shown to be highly bioavailable.<sup>47</sup> The bioavailability of natural astaxanthin can be increased by taking astaxanthin meal or extract with a meal containing fats. There is not much information in the literature regarding the pharmacokinetics of oral astaxanthin in humans. However, in an open parallel study, healthy male volunteers received a single dose of 40 mg astaxanthin, as lipid based formulations, 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.<sup>68</sup>

Product information

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