Safety Profile

Background

BioAstin® is a natural extract of dried *Haematococcus pluvialis* microalgae, rich in the red carotenoid pigment astaxanthin. A growing body of scientific literature is demonstrating that dietary astaxanthin has profound antioxidant potential and beneficial effects on health. *Haematococcus* microalgae are cultivated for the production of BioAstin® at Cyanotech Corporation's oceanside facility in Kona, Hawaii (Lorenz and Cysewski 2000). BioAstin® is processed according to Good Manufacturing Practices (GMP) under an ISO 9002 certified quality management system. *Haematococcus* has never been associated with any toxicity in the reported literature or in field studies, and numerous animal and human studies lend support to its safety (Maher 2000). *Haematococcus pluvialis* algae has been reviewed by the US Food and Drug Administration (1999; Docket No. 95S-0316) and cleared for marketing as a new dietary ingredient by means of the Dietary Supplement Health and Education Act (DSHEA). It is also been approved in Japan for use in both foods and animal feeds. A different formulation of *Haematococcus* algae has already gained wide acceptance in the aquaculture markets as a pigmentation and vitamin source for salmon, trout, shrimp and ornamental fish and has been approved as a feed additive for salmonids by the Canadian Food Inspection Agency and the US Food and Drug Administration. Similar registrations are in progress in the European Union and other countries.

Natural Astaxanthin in the Human Diet

Astaxanthin is common in nature, especially in the marine environment. It is probably best known for producing the pinkish-red hue of the flesh of salmon and trout, as well as of shrimp, lobsters and crayfish. These animals cannot synthesize astaxanthin, so they must obtain it from their diet, which includes zooplankton that have ingested astaxanthin-containing algae. A recent study assessed the astaxanthin concentration in a variety of wild salmonid species. This survey showed a range of astaxanthin concentrations in the flesh from 1-58 mg/kg (Turujman et al. 1997). The results are summarized in Table1 with the calculated average concentrations of each species and as all species combined.

TABLE 1

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 all species		**13.2 mg/kg**

It can be seen from Table 1 that the average astaxanthin concentration ranges from 5.3 mg/kg in Atlantic salmon to 40.4 mg/kg in sockeye salmon. The average of all species was calculated to be 13.2 mg/kg. Since the average human would consume about 0.25 kg of fish flesh in one meal, this results in the lowest intake of 1.3 mg of astaxanthin from Atlantic salmon, 3.3 mg of astaxanthin from "average" salmon and 10.1 mg of astaxanthin from sockeye salmon. Recommended dosages of BioAstin[®] natural astaxanthin (4-12 mg daily) are therefore comparable to the astaxanthin consumed in a single salmon meal.

Other natural carotenoid pigments are found in BioAstin[®], including canthaxanthin, lutein and beta-carotene, but they are present at less than 5% of the level of astaxanthin. These carotenoids are commonly found in fruits and vegetables of the normal human diet. Total canthaxanthin, lutein and beta-carotene intake from BioAstin[®] would be less than 0.6 mg/day. Canthaxanthin is currently allowed for use in coloring foods under the US Code of Federal Regulations (CFR 21 section 73.75) at levels not to exceed 30 mg per pound of food or pint of liquid food. Thus, the canthaxanthin ingested from the recommended dose of BioAstin[®] is 50 to 150-fold less than would be ingested from a pound of food or pint of liquid colored with canthaxanthin. The usual recommended doses of beta-carotene and lutein are 20-60 mg/day and 3-6 mg/day, respectively.

Natural astaxanthin has been marketed in the US, Europe and Japan in various forms for many years. Itano Refrigerated Food Co. Ltd. (Tokushima, Japan) markets astaxanthin from extracted Antarctic krill as a human supplement. This product is distributed by the U.S. company, OptiPure, as "Astax-1700[™]". IGENE Biotechnology Inc. (Columbia, Maryland) produces natural astaxanthin from the yeast *Xanthophyllomyces dendrorhous* (formerly *Phaffia rhodozyma*), which is marketed for human consumption as "AstaXin[®]." Several astaxanthin products from *Haematococcus* algae are presently marketed as dietary supplements, including "BioAstin[®]" (Cyanotech Corp., Kona, Hawaii), "AstaFactor[®]" (Mera Pharmaceuticals, Inc., Kona, Hawaii), and "AstaREAL[®]" (Fuji Chemical Industry Co. Ltd., Toyama, Japan).

Safety Studies – Human

A randomized, double-blind, placebo-controlled human safety study was recently conducted with BioAstin[®] natural astaxanthin (Spiller 2002). Thirty-five healthy adults took 3 gelcaps per day for eight weeks. Nineteen subjects received a placebo containing only safflower oil, while 16 received the algae extract mixed in safflower oil (2 mg astaxanthin per gelcap). 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 ($P<0.05$) were detected between the treatment and placebo groups after eight weeks of supplementation, except for serum calcium, total protein and eosinophils. Though statistically significant, these few differences were slight and of no clinical importance. The study concluded that 6 mg of astaxanthin per day from BioAstin[®] can be safely consumed by human adults.

In another reported human safety trial (Aquasearch Inc. 1999a), 33 adults consumed either 3.85 mg astaxanthin (low dose) or 19.25 mg astaxanthin (high dose)

daily, in the form of tabletted *Haematococcus* algae meal, for four weeks. Comprehensive blood tests, urinalyses and physical examinations were carried out at the beginning of the trial, after three to seven days of supplementation, and at the end of the four week supplementation period. No changes of any clinical significance were noted after supplementation with either dosage of *Haematococcus* astaxanthin.

Other Human Studies

A number of efficacy studies involving human consumption of astaxanthin products have been carried out in recent years; although not specifically designed as safety studies, they support the conclusions of the safety trials, and further, some indicate potential therapeutic properties of dietary supplementation with astaxanthin.

Randomized, double-blind, placebo-controlled human clinical trials have been completed which tested the efficacy of BioAstin[®] in the relief of pain and the improvement of performance in patients with carpal tunnel syndrome (CTS) and rheumatoid arthritis (RA). These clinical trials were initiated after individuals suffering from CTS reported receiving relief from their symptoms after including BioAstin[®] supplements in their diet (Lorenz and Cysewski 2001). In the first study (Nir et al. 2002a), 13 adults suffering from CTS were given BioAstin[®] gelcaps (12 mg astaxanthin/day) and 7 received a placebo. Daytime pain rate and duration were assessed by questionnaire at 0, 4 and 8 weeks. At both 4 and 8 weeks of the trial, individuals in the treatment group reported reduced rate and duration of pain compared to the control group. Although the results were not statistically significant due to the small number of subjects, there was a trend toward reduced pain with BioAstin[®] supplementation. The second study (Nir et al. 2001b) involved 21 patients suffering from RA; 14 received BioAstin[®] (12 mg astaxanthin/day) and 7 received a placebo. Questionnaires were used to assess pain and satisfaction with the ability to perform usual activities at 0, 4 and 8 weeks of supplementation. At the end of the study, pain was reduced and satisfaction was increased in the treatment group relative to the control group. These results were statistically significant ($P<0.05$).

Two studies of the effects of astaxanthin on human muscles have recently been carried out. In the first (Malmsten 1998; see also Lignell 2001), the effect of natural astaxanthin on muscle endurance was tested on forty young healthy male students. The students were divided into two groups, half receiving astaxanthin and half a placebo. The astaxanthin (4 mg daily) was administered as a capsule filled with *Haematococcus* algae meal. After six months of supplementation, a significant ($P<0.05$) improvement in muscle strength and endurance (as assessed by knee bends) was noted in the treatment group relative to the control group. No differences in blood hemoglobin, steady state pulse or subjective assessment of well-being were found between the treatment and placebo groups. In the second study (Fry 2001), twenty weight-trained males were recruited to test the effect of BioAstin[®] supplementation on delayed onset muscle soreness (DOMS). In this randomized, double-blind study, ten subjects received BioAstin[®] (2 mg astaxanthin) daily and ten received a placebo daily for three weeks. At the end of the supplementation period, the subjects conducted a vigorous exercise session designed to induce DOMS. Strength assays, pain questionnaires and comprehensive blood analyses were carried out pre-supplementation, immediately before the DOMS

session, and at several time points following the session (up to twelve days). Although no clear improvement in DOMS was noted in the study, neither was there any noted effect of supplementation with BioAstin® on blood chemistry, except for a slight elevation of immune response (white blood cells and neutrophils) relative to the placebo group following the DOMS exercise session. The study concluded that there appeared to be no health-related problems associated with taking the prescribed dosage of BioAstin®.

In the spring of 2001, Mera Pharmaceuticals Inc. surveyed 758 known users of their AstaFactor® natural microalgal astaxanthin supplement regarding the efficacy of astaxanthin at relieving their specific conditions (Guerin et al. 2002). From the 247 respondents to the survey there was a total of 328 health conditions reported; astaxanthin supplementation was reported to have improved 85% of these conditions. Although these results are highly subjective and not controlled, they do suggest that regular users of natural astaxanthin are finding it to be beneficial to their health. Moreover, 6 of 7 respondents with rheumatoid arthritis and 19 of 20 with osteoarthritis claimed an improvement in their condition from taking astaxanthin supplements, which is consistent with the results of the BioAstin® human clinical trial on RA cited above. Other such qualitative findings of potential health benefits associated with BioAstin® supplementation include reports by individuals of the retardation and prevention of sunburn (Lorenz 2002b) and the retardation and amelioration of fever blisters and canker sores (Lorenz 2002a).

In a study of human metabolism of astaxanthin, three healthy adult male volunteers each ingested a single dose of 100 mg astaxanthin (Østerlie et al. 2000). This dietary astaxanthin was readily absorbed and incorporated into plasma lipoproteins and metabolized over time; no ill effects on the subjects were noted. Although the astaxanthin used in the study was synthetic and the study was not controlled, the lack of any negative effects suggests that humans are not adversely affected by large single doses of astaxanthin (in this case, 8-25 times the recommended daily dose from BioAstin®).

Safety Studies – Animal

Astaxanthin was approved for use in salmonid feeds under 21 CFR section 73.35 up to a level of 80 mg/kg, after a successful Color Additive Petition (CAP 7C0211; F. Hoffmann-La Roche & Co. Ltd. 1987) was reviewed by the FDA. This petition included numerous safety studies with astaxanthin (synthetic).   Volume 2 of this petition contains the summaries from the studies. The acute toxicity of 10 consecutive daily oral doses astaxanthin in rats was found to be greater than 2000 mg/kg. There was no mortality or symptoms of toxicity reported. In the Ames mutagenicity test, astaxanthin concentrations ranging from 0.03-5.0 mg/plate did not induce mutations in *Salmonella typhimurium* tester strains with or without activation by rat liver homogenate. Astaxanthin administered to mice at 500, 1000, and 2000 mg/kg did not induce chromosome breaks or mitotic disjunction. In teratology and embryotoxicity studies with rabbits, doses ranging from 100-400 mg/kg/day were administered to pregnant animals. There were neither overt signs of maternal sensitivity to the treatment nor significant changes in body weight development or malformations among the fetuses compared to the controls.   Other safety studies included reproductive performance in rats with P and F1 generations, a 13-

week tolerance study in rats, and a 13-week tolerance study in dogs; none revealed toxic effects. The full volume of these safety studies is available in CAP 7C0211 at the FDA.

A number of standard toxicity and safety studies have been conducted with *Haematococcus* algae. An acute oral toxicity study was conducted on Charles River CD rats with a single dose of 5 g/kg of *Haematococcus* algae and observation for 13 days (International Research and Development Corp. 1989). Groups were evaluated for mortality, pharmacotoxic signs, and body weights during, and given necropsy examinations following the study. The results demonstrated that the LD_{50} value of each lot was greater than the administered dose of 5 g/kg. No visible abnormalities were observed, nor differences in body weights during the study. The postmortem examination did not reveal any abnormalities in rats sacrificed at the end of the study.

Another acute toxicity study was conducted in which rats were administered 12 g/kg of *Haematococcus* algae orally in a single dose (Istituto di Ricerche Biomediche "Antoine Marxer" RBM S.p.A. 1995). At the end of the 14-day observation period, there were no mortalities, adverse clinical signs or behavioral alterations noted in the animals. Body weight gain was unaffected by the treatment and a post-mortem pathology showed no appreciable macroscopic findings at the end of the 14 days. It was concluded that the LD_{50} value was higher than 12 g/kg. An oral toxicity study was conducted at the same laboratory in which rats were administered 6 g/kg of *Haematococcus* algae daily for 14 days (Istituto di Ricerche Biomediche "Antoine Marxer" RBM S.p.A. 1996). No treatment-related deaths occurred during the course of the study. Routine clinical and laboratory observations did not show any adverse changes in the test animals of either sex. The post-mortem examination showed no changes in organ weight or gross pathology. It was concluded that *Haematococcus* algae administered by oral route at the maximum dosage of 6 g/kg/day was well tolerated and caused no adverse effects.

In Japan, higher dosage studies of acute oral toxicity have been conducted with both male and female mice fed single doses of *Haematococcus* algae ranging from 10.4-18.0 g/kg (Nippon Animal Feeding Corp. 1988). No mortalities or abnormalities were observed at the end of the study. Mutagenicity tests conducted on the same algae meal were also negative (Japan Food Analysis Center 1988).

A published study with rats fed 400 mg/kg astaxanthin for 41 days showed no harmful effects on body/organ weight, enzyme activities, pregnancy, or litter size (Nishikawa et al. 1997). In this study, three forms of astaxanthin were tested: synthetic astaxanthin, *Haematococcus* algae, and *Xanthophyllomyces* (*Phaffia*) yeast. No toxicity was noted from any of these astaxanthin sources.

Recently, the preliminary results of a 28-day *Haematococcus* algae repeated-dose oral toxicity study in rats were reported (Aquasearch Inc. 1999b). Three groups of 20 rats (10 male, 10 female) each were employed: a control group, a low dose group and a high dose group. The low dose was 5 mg/kg and the high dose was 50 mg/kg of algae meal; both were administered orally to the rats daily. The administration of *Haematococcus* algae to the rats at either dosage did not cause mortality or an increase in adverse system observations when compared with the control group.

A 13-week oral repeated-dose toxicity study of *Haematococcus* astaxanthin was conducted at the Japanese National Institute of Health Science (Ono et al. 1999). F344 rats were administered daily a *Haemotococcus* algae extract sprayed onto feed. Different concentrations of the extract, ranging from 0-0.25% of the feed, were tested. After 13

weeks of testing, there were no exposure-related changes, adverse effects, or toxicological effects noted in the treatment groups relative to the control group. A similar study at the same institute using *Xanthophyllomyces* (*Phaffia*) yeast as a source of astaxanthin also found no toxicological effects in rats (Onodera et al. 1997).

Other Animal Studies – Mammals and Birds

Numerous targeted studies have been published over the past decade in which astaxanthin has been administered to mice and rats. In a series of in vivo studies at the Gifu University School of Medicine in Japan, dietary astaxanthin was found to be a potentially effective chemopreventative agent against carcinogenesis of the mouse urinary bladder (Tanaka et al. 1994), rat oral mucosal tissues (Tanaka et al. 1995a) and rat colon (Tanaka et al. 1995b). Another in vivo study found dietary astaxanthin to be more effective than canthaxanthin or beta-carotene at inhibiting the growth of transplantable mammary tumors in mice (Chew et al. 1999a); these researchers at Washington State University also found that astaxanthin stimulated splenic lymphocyte function in mice (Chew et al. 1999b).

Several in vivo demonstrations of the potential for astaxanthin to enhance the immune response in mice have been published. Dietary astaxanthin delayed the onset of lymphadenopathy and proteinuria in autoimmune-prone mice (Tomita et al. 1993) and enhanced specific antibody production to T-dependent antigens in B6 mice (Jyonouchi et al. 1994). Astaxanthin supplementation also improved the immunological dysfunction and markedly attenuated the promotion of hepatic metastasis induced by restraint stress in mice (Kurihara et al. 2002; see also Asami et al. 2001). Dietary astaxanthin also stimulated immunity against methylcholanthrene-induced tumors in mice; this immune stimulation was associated with suppression of tumor growth (Jyonouchi et al. 2000).

Astaxanthin is a potent antioxidant in vitro, and studies with rats and mice have confirmed that it has antioxidant properties in vivo as well, protecting biological membranes from oxidative damage. In vitamin E-deficient rats, dietary astaxanthin protected the mitochondria from damage by Fe^{2+}-catalyzed lipid peroxidation, and inhibited carrageenan-induced inflammation of the paw (Kurashige et al. 1990). Astaxanthin also reduced lipid peroxide levels of rat liver following intraperitoneal administration of CCl_4, and reduced lipid peroxide levels within rat serum, liver, kidney, spleen and brain following exposure of the animals to ^{60}Co radiation (Nishigaki et al. 1994). Astaxanthin inhibited putrescine accumulation in the skin of harless mice following exposure to UVA plus UVB radiation (Savouré et al. 1995). An astaxanthin-rich extract of *Haematococcu*s algae has proven effective at reducing gastric inflammation associated with *Helicobacter pylori* infection in mice, apparently through both its inhibition of lipid peroxidation and through an inhibitory effect on *H. pylori* growth (Bennedsen et al. 1999; Wang et al. 2000; see also Wadström and Alejung 2001).

In tests with male albino Lewis rats, natural astaxanthin from krill and yeast sources was found to be effective at protecting the retina from photic injury, and also at ameliorating the effects of such photic injury to retinal neurons and other cells (Tso and Lam 1996). This rat study also demonstrated the ability of astaxanthin, but not beta-carotene, to cross the retinal blood-brain barrier, suggesting that astaxanthin might be capable of providing a protective effect on other parts of the central nervous system. No

tendency for astaxanthin to crystallize in the retina was noted. In another eye health study, an astaxanthin-containing algal extract attenuated selenite-induced nuclear cataract formation in rat pups (Wu et al. 2002).

The effect of dietary astaxanthin on serum cholesterol levels of rats has also been investigated (Murillo 1992). Male Wistar rats fed 0.1% dietary astaxanthin for 30 days had increased HDL ("good") cholesterol (57 mg/dL) in their serum compared to rats fed the control diet (42.4 mg/dL).   Conversely, the LDL ("bad") cholesterol was higher in rats fed the control diet (12.5 mg/dL) than in those fed a diet supplemented with astaxanthin (9.6 mg/dL). No adverse reactions were reported in the study.

A recent study reported beneficial effects of astaxanthin supplementation on type 2 diabetic mice (Uchiyama et al. 2002). Astaxanthin-treated mice, relative to the non-treated group, showed lower non-fasting blood glucose levels, preserved islet cell function, and reduced renal dysfunction (as measured by 8-OhdG and albumin levels) after 12 weeks of supplementation.

A number of reports have been made of the successful use of astaxanthin to promote health in livestock . Natural astaxanthin from *Haematococcus* algae has been used to treat exertional rhabdomolysis in horses (Lignell 2001) and mastitis in cows (Lignell and Inborr 2002a), and to enhance the production of immunoglobulin-rich milk in cows (Lignell and Inborr 2002b). *Haematococcus* astaxanthin is also reported to enhance semen production and reproductive performance in boars (Lignell et al. 2002), and to enhance the production results of both breeding hens and their chickens (Lignell et al. 1998).

Other Animal Studies – Fish and Crustaceans

Astaxanthin has a long history as a feed additive for aquacultured fish and crustaceans. In addition to its role as a coloring agent, it has been found to have metabolic functions in these animals. Results of feeding trials of rainbow trout have not revealed any adverse effects of astaxanthin on the fish, regardless of whether the astaxanthin source was synthetic, yeast-based or from *Haematococcus* algae, and in some cases improvement in certain biochemical characteristics of the liver and blood resulted from the astaxanthin administration (Nakano et al. 1995, 1999; Řehulka 2000; Hardy 2001). Similarly, carotenoids (especially astaxanthin) are considered important antioxidant "vitamins" for the health of farmed Atlantic salmon (Torrissen 1990; Torrissen and Christiansen 1995; Bell et al. 2000). Astaxanthin also improves spawning performance and egg production in striped jacks (Vassallo-Agius et al. 2001a, 2001b), and improves stress resistance and survival rate in prawns (Chien and Jeng 1992; Merchie et al. 1998; Chien et al. 2003). No negative effects of astaxanthin supplementation on animal health were noted during any of these studies.

In Vitro Properties of Astaxanthin

Due to its particular molecular structure, astaxanthin serves as an extremely powerful antioxidant (Terao 1989; Jørgensen and Skibsted 1993; Goto et al. 2001). In *Haematococcus* microalgae, astaxanthin naturally protects cells from oxidative damage, including that induced by UVB radiation (Kobayashi and Okada 2000). Protection by

astaxanthin from UVA light-induced oxidative stress has also been demonstrated in vitro in cultured rat kidney fibroblast cells (O'Connor and O'Brien 1998). Recently, an astaxanthin-rich algal extract exhibited similar protective properties from UVA damage in various human cell lines (Lyons and O'Brien 2002). Astaxanthin was also shown to effectively protect chicken embryo fibroblasts against paraquat-induced oxidative stress (Lawlor and O'Brien 1995).

Astaxanthin, unlike beta-carotene, is a "pure" antioxidant: that is, it exhibits no prooxidant potential even at high concentration and high oxygen tension (Martin et al. 1999; Beutner et al. 2001). Astaxanthin has been shown to be particularly effective at protecting lipid membranes from peroxidation (Palozza and Krinsky 1992; Lim et al. 1992; Naguib 2000; Rengel et al. 2000). The in vitro oxygen free radical scavenging ability of BioAstin® natural astaxanthin has been tested and was found to be superior to that of vitamins C and E, beta-carotene and all-*trans*-retinol (Bagchi 2001). These results add to the growing body of literature supporting the role of astaxanthin in protecting cells and organisms from oxidative damage (e.g., Miki 1991; Tinkler et al. 1994; Shimidzu et al. 1996; Figure 1).

In addition to its antioxidant properties, astaxanthin has exhibited immunomodulating properties in a variety of animal cell lines (Jyonouchi et al. 1991, 1993, 1995b, 1996; Okai and Higashi-Okai 1996). These properties do not relate to vitamin A activity and are not clearly related to antioxidant activity. Immunomodulating properties of astaxanthin have also been demonstrated in human blood cells (Jyonouchi et al. 1995a). In vitro studies with *Haematococcus* astaxanthin have also indicated an anti-proliferative effect on prostate cancer cells and an inhibitory effect on the activity of the 5α-reductase enzyme associated with benign prostate hyperplasia (Anderson 2001).

Figure 1. Relative antioxidant potential of carotenoids and alpha-tocopherol (vitamin E). Adapted from Shimidzu et al. 1996.
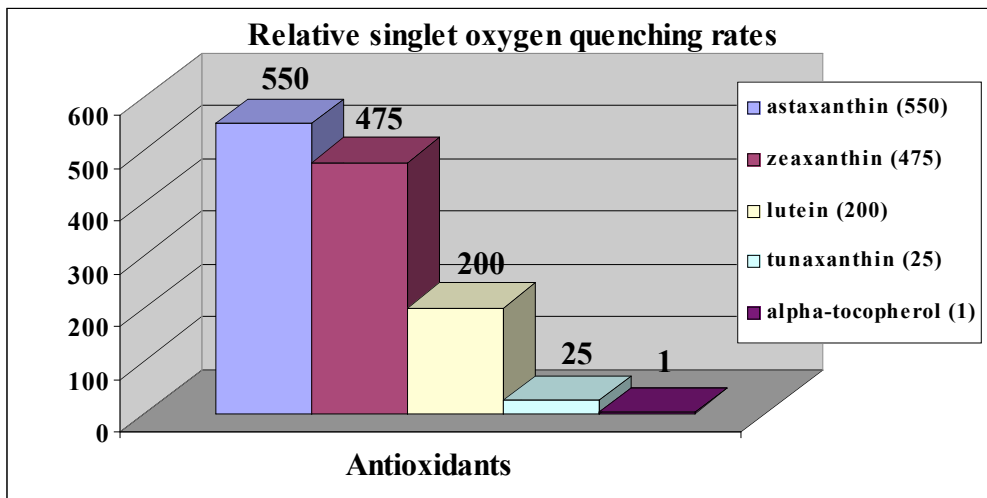
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