Astaxanthin: Frequently Asked Questions

1. *What is astaxanthin?*

Astaxanthin is a carotenoid produced in microalgae, yeast, bacteria and fungi; in the aquatic environment microalgae are consumed by zooplankton, insects or crustaceans (e.g. krill, shrimp, lobster and crayfish) which accumulate astaxanthin and in turn are ingested by fish (e.g. salmon and trout). Astaxanthin cannot be produced by humans; it has to be taken up – like the other carotenoids – via the diet or supplements. Astaxanthin is a very strong antioxidant; potential applications in humans build on the antioxidant function. Astaxanthin can be produced via fermentation or by synthesis. Products produced with one or the other technology are available on the market.

Astaxanthin has been used in animal nutrition as a pigment in the aqua-culture industry; for human applications both esterified or free astaxanthin can be used. The esterified astaxanthin predominantly comes from production via microalgae while the "non-esterified" or free astaxanthin comes from other forms of production. Esterified astaxanthin is then "de-esterified" in the gut before it is taken up in the blood. As a result of its antioxidant potential, astaxanthin is associated with several health benefits e.g. heart health, skin health and benefits for the immune system.

Astaxanthin naturally occurs as a part of the diet;. Although exposure may vary according to the diet consumed because it is only contained in relevant concentrations in a few food items. Wild-type salmon on average contains about 0.5-1.25 mg of astaxanthin per 200 g salmon (Rufer et al. 2008). Since most Americans consume salmon only up to two-three times per week, it is likely that dietary intakes of astaxanthin are relatively low compared to the doses in studies demonstrating the safetyof astaxanthin.

The package of safety studies undertaken with DSM manufactured astaxanthin is very comprehensive. The package included general toxicity studies in rodent and non-rodent species, developmental reproductive toxicity studies and carcinogenicity studies in the rat and mouse. In these studies, the lowest No Observed Adverse Effect Level (NOAEL) was 40 mg/kg bw/day.

An Acceptable Daily Intake (ADI) is conventionally derived using a 100 fold safety factor applied to the lowest NOAEL in the longest and/or more sensitive toxicity study. In the case of DSM astaxanthin (using 40 mg/kg bw/day) this provides an ADI intake of 0.4 mg/kg bw/day for man. For a 60 or 70 kg person this equates to 24 or 28 mg/day, respectively. The DSM proposed supplement dosage is well below the calculated safe ADI dosage. Indeed, clinical studies demonstrating potential benefits of astaxanthin have used a range of astaxanthin starting at 2 mg/d to as high as 22 mg/d, depending on the health outcome that was evaluated.

2. *Bioavailability and metabolism*

As demonstrated in the available clinical literature, we do know that both natural and nature-identical astaxanthin are bioavailable. It has been reported that the bioavailability of astaxanthin in humans as measured by blood levels, is not affected by the stereoisomeric composition of astaxanthin. For example: a 28 days human study by Rufer et al.(2008) showed that plasma astaxanthin levels were higher in individuals eating aqua-cultured salmon (fed with nature-identical astaxanthin) compared with those eating wild-type salmon at equivalent astaxanthin doses (1.25 mg/d). Moreover Osterlie et al (2000) and Coral-Hinostroza et

al (2004) both showed bioavailability for the nature-identical form of astaxanthin. In fact, both studies measured plasma concentrations of astaxanthin greater than the one demonstrated by Okada et al. (2009) who supplemented humans with natural astaxanthin derived from Haematococcus pluvialis.

In view of nature-identical astaxanthin, which comprises a mixture of all three stereoisomers, indeed clinical data demonstrate that it can be measured in the bloodstream of humans as quickly as 2 hours after oral supplementation. Also in humans it can be measured in lipoproteins, e.g. VLDL, LDL and HDL, which are typically the sites of action for astaxanthin. Astaxanthin incorporated into these lipoproteins can act as an antioxidant to reduce the oxidation state of these lipoproteins and so support heart health. There are clinical studies that support the heart health benefit of astaxanthin through this mechanism of action (*Iwamoto et al. 2000, Yoshida et al. 2010; Miyawaki et al. 2008; Kim 2004).*

Furthermore, in comparison to the bioavailability data of free astaxanthin, Coral-Hinostroza et al. (2004) showed that esterified astaxanthin had a lower bioavailability in humans. It was speculated that this was related to the requirement for hydrolysis before uptake, which may limit the amount available at the site of uptake. Therefore, as for other xanthophylls (lutein, zeaxanthin), the ester form must be hydrolyzed before absorption and before a biological benefit can be exerted.

3. *Efficacy*

From a clinical efficacy perspective, astaxanthin has been shown to exert heart health benefits primarily because of its role as an antioxidant whereby it decreased oxidation of LDL and VLDL. Indeed, the studies evaluating the bioavailability of nature-identical astaxanthin in humans show significant accumulation of astaxanthin not just in plasma but more specifically in LDL, VLDL and HDL, after astaxanthin consumption (Osterlie et al 2000; Coral-Hinostroza et al 2004).

4. *What are the isomeric forms that are present in natural and nature-identical astaxanthin?*

Astaxanthin can – due to the two stereo centers - exist in three isomeric forms, (3S,3S'), (3R,3S') and (3R, 3R'), depending on the spatial orientation of the hydroxyl groups in chiral carbon number 3 and 3'.

Analysis of a variety of fish and crustacean species demonstrates that all three isomeric forms co-exist in nature (Molkentin J. et al. 2012; Higuera-Ciapara et al. 2006). Indeed, a study by the FDA showed that in wild Pacific and Atlantic salmon there were small amounts of the (3R,3S') and (3R,3R') forms present, along with the 3S,3S' isomer (Rufer et al. 2008).

Nature-identical astaxanthin consists of all three isomeric forms, (3S,3S'), (3R,3S') and (3R,3R') in a 1:2:1 ratio (Higuera-Ciappara et al. 2006). As described in the DSM's Product Data Sheet, DSM's AstaSana astaxanthin also comprises all three isomers, (3S,3S'), (3R,3S') and (3R,3R') in a 1:2:1 ratio.

What are the isomeric forms present in competitor's product (i.e. Fuji, Cyanotech, Algaetech)?

For those forms of astaxanthin derived from *Haematococcus pluvialis,* astaxanthin comprises only the (3S,3S`) enantiomer. Astaxanthin derived from yeast *Phaffia* exists in the (3R,3R') enantiomeric form. All three enantiomeric forms have been reported to occur in nature, especially in fish and crustacean species (Molkentin J. et al. 2012; Higuera-Ciapara et al. 2006).

Is there scientific evidence that suggests which isomeric forms are more efficacious?

With respect to astaxanthin, differences in bioactivity or efficacy as a result of differences in stereo-chemistry for astaxanthin at the in-vitro or in-vivo level have not been demonstrated nor confirmed. In other words, there is no scientific evidence (pre-clinical or clinical) to support that the antioxidant benefit or any other health benefit of astaxanthin can be ascribed to a specific stereoisomer present in astaxanthin. This point is substantiated by data from Lockwood and Gross (2005).

Herein, the authors tested the antioxidant potency of nature-identical astaxanthin, a mixture of the stereoisomers (3S,3S'), (3R,3S') and (3R,3R') in a 1:2:1 ratio and the individual stereoisomers (of nature-identical astaxanthin). The authors used the activated human neutrophil assay to measure the superoxide radical scavenging activity. The assay demonstrated that the nature-identical astaxanthin at millimolar concentrations, was capable of nearly completely eliminating the superoxide anion signal generated, and that there was no significant difference in scavenging efficiency among the individual stereoisomers and the mixture of stereoisomers (Lockwood and Gross, 2005).

The authors also measured the efficacy of this nature-identical astaxanthin containing the mixture of stereoisomers [(3S,3S'), (3R,3S') and (3R,3R') in a 1:2:1 ratio] in rats, dogs and rabbits, and showed protection against experimental mycocardium injury as well as an anti-inflammatory benefit in rabbits (Lockwood and Gross 2005).

How does DSM's astaxanthin isomeric profile fit into that of the scientific studies, how do we position that our product is efficacious and that our ingredient is bioavailable?

There is no scientific evidence (pre-clinical or clinical) which supports the antioxidant benefit, or any other health benefit, as a result of astaxanthin supplementation, that can be ascribed to a specific stereoisomer of astaxanthin. Data from Lockwood and Gross (2005) demonstrate that all stereoisomers of nature-identical astaxanthin are capable of antioxidant activity.

Is there any stereoisomer conversion in a biological system?

In regard to in vivo stereoisomer conversion within biological systems, the studies of both Rufer et al. (2008) and Osterlie et al. (2000) report that there is no appreciable metabolic transformation of astaxanthin after consumption, and that the optical isomer distribution in plasma resembled that of the dietary dose provided.

Is there any benefit of the esterified vs. free form of astaxanthin?

With respect to the free and esterified forms of astaxanthin, while the esterified form is reported to be the most predominant in nature, the free form can also be found (Higuera-Ciapara et al. 2006); for example, Foss et al 1987 reported the presence of the free form of astaxanthin in salmon. Regardless of the supplementation form of astaxanthin (free vs. esterified), the free form is the only form found in human blood after supplementation with either esterified or unesterified nature-identical and natural astaxanthin (Okada et al. 2008; Osterlie et al. 2000; Coral-Hinostroza et al. 2004). Furthermore, it has also been reported from studies in humans, that the esterified form of astaxanthin has a lower bioavailability than the unesterified free-form. Coral-Hinostroza et al. (2004) showed that esterified astaxanthin has a lower bioavailability in humans. It is speculated that this is related to the requirement for hydrolysis before uptake, which may limit the amount available at the site of uptake. Therefore, as for other xanthophylls (lutein, zeaxanthin), the ester form must be hydrolyzed in the gastro-intestinal track prior to absorption

and before any biological benefit can be exerted. Lockwood and Gross (2005) further confirmed that the non-esterified form of astaxanthin is believed to be the primary active form.

5. *Summary*

DSM's AstaSana™ brand astaxanthin is supported by the most extensive safety data set specific to any single astaxanthin product currently available in the marketplace. DSM's Astaxanthin has moreover undergone a formal approval by the Food and Drug Administration as an article of commerce in the US food supply, unlike many of our competitors in the natural astaxanthin space. The safety studies performed by DSM for our astaxanthin product, as a part of the public record, have even been referenced as the basis for safety for other commercially available astaxanthin (including natural) producers in the market today. There is emerging evidence supporting the use of astaxanthin for human applications, e.g. support of heart health, immune benefits, skin and eye health at doses ranging from 2-22 mg/d. This level of use is consistent with the ADI calculated by DSM using DSM's astaxanthin safety data. DSM's AstaSana is a valuable addition to the US dietary supplement market, providing a potent and bioavailable product with a reliable supply chain.

6. *References:*

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