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Review of genotoxicity and rat carcinogenicity investigations with astaxanthin

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ABSTRACT

Synthetic astaxanthin has been extensively tested for safety. Genotoxicity studies including Ames and in vitro Micronucleus Tests show absence of genotoxic potential. Although a long-term mouse study showed no carcinogenicity potential, the rat carcinogenicity study with dietary dosages of 0 (control), 0 (placebo beadlet), 40, 200 and 1000 mg astaxanthin/kg bw/day showed an increased incidence of benign, hepatocellular adenoma in females only, at 200 mg/kg bw/day and above. There was no clear evidence of toxicity during the in-life phase. Discoloration of feces was observed and a reduction in body weight gain in all groups receiving beadlets, probably reflecting a nutritional influence. Blood sampling confirmed systemic exposure and some minor clinical chemistry differences in females at 200 and 1000 mg/kg bw/day. There was no effect on adjusted liver weight. Histopathological examination showed hepatic changes indicative of slight hepatotoxicity and hepatocyte regeneration in females at 200 and 1000 mg/kg bw/day, in addition to the adenoma. Taking into account this pathological background in the female rat, and a wide variety of other supporting information, it is concluded that the hepatocellular adenoma in female rats was secondary to hepatotoxicity and regeneration, and is most probably a species-specific phenomenon of doubtful human relevance.

1. Introduction

Astaxanthin (CAS No. 472-61-7) is a naturally occurring xanthophyll (Fig. 1) that is consumed from dietary sources and principally from eating salmon. Three optical isomers exist in nature in variable ratios while the synthetic material is usually a racemic mixture of 3S,3′S: 3R,3′S: 3R,3′R in a ratio of 1:2:1 (Moretti et al., 2006). The 3R,3′S isomer is sometimes referred to as the meso form. Astaxanthin also has various geometrical isomers with all-trans astaxanthin (all-E astaxanthin) as the predominant one. In addition, at least two other geometrical cis-isomers (Z-isomers) occur to some extent in nature and in racemic astaxanthin from synthesis.

Wild salmon obtain their astaxanthin from the food chain but farmed salmon need to receive astaxanthin supplementation in their feed to give the characteristic color of salmon flesh.

The safety to humans of the dietary consumption of astaxanthin from salmon, whether wild or farmed, has been affirmed by various regulatory authorities.

Astaxanthin apart from its color has been shown to have various beneficial protective qualities and there is increasing interest in the direct human supplementation to achieve an intake above the intake achieved from salmon consumption. This raises again the question as to what is the safe upper intake of astaxanthin. Many toxicological studies have been undertaken with astaxanthin in laboratory species in the past and have been reviewed recently (EFSA, 2014a). It has been shown that astaxanthin is not genotoxic and can be safely consumed at high intakes with no adverse impact on reproduction or longevity. In mammals astaxanthin is not considered to be a pro-vitamin A carotenoid (Astorg et al., 1997) as retinol is not a product of direct metabolic cleavage of astaxanthin.

Short term studies for genotoxicity performed in the past have shown absence of genotoxic potential.

Chronic toxicity and carcinogenicity of astaxanthin have been investigated in a series of standard animal toxicity studies, comprising a 1-year chronic toxicity study in rats, a 2-year carcinogenicity study in rats (Buser et al., 2003a), an 18-month

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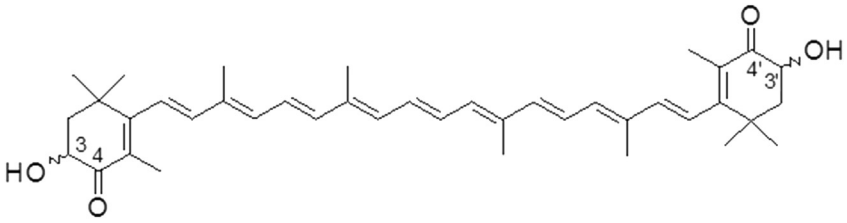

Fig. 1. Chemical structure of all-trans astaxanthin.

carcinogenicity study in mice, and a 1-year chronic toxicity study in dogs. In the 18-month mouse carcinogenicity study (Buser et al., 2003b), the authors concluded that astaxanthin via the dietary route was well tolerated and there was no evidence for an oncogenic potential of astaxanthin in mice at doses up to 1400 mg/kg bw/day (the highest dosage). Also there was no evidence of hepatic toxicity. In the dog study (Bremer, 1995) there was no evidence of hepatic toxicity at the highest dose level of 1200/2500 mg/kg bw/day (first five months 1200 mg/kg bw/day, ensuing seven months 2500 mg/kg bw/day). In a 1-year rat study (Buser et al., 2003c) in female rats, there was a low magnitude (<2-fold) increase in plasma enzymes (aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase) and an increase in centrilobular hepatocellular hypertrophy at dosages of 250 mg/kg bw/day and above, but not at the incrementally lower dosage of 125 mg/kg bw/day. Transaminase enzymes, although not specific for liver toxicity, are commonly increased when there is hepatotoxicity.

The hepatotoxicity seen in female rats in the 1-year and carcinogenicity studies (which also was not associated with an adverse impact on longevity) is regarded as the most sensitive end-point for an adverse effect of astaxanthin in all the safety studies previously performed with astaxanthin in the mouse, rat and dog. Following standard, conservative toxicological principles (precautionary principle) the most sensitive endpoint (liver findings in female rats) has been used as the basis for defining the safe level of human intake.

Key data from the rat carcinogenicity study, which was undertaken over a decade ago, is publically available via the FOI (Freedom of Information Act) in the USA, following previous FDA evaluations, and through publically available Scientific Opinions from EFSA. Further publication of this data here is to meet the common knowledge element of the GRAS (Generally Regarded as Safe) standard, which requires that the primary evidence of safety be generally known and accepted.

The genotoxicity studies and the two-year rat carcinogenicity study are important elements of the overall package of safety studies performed for astaxanthin and therefore are being made publically available, in addition to the studies published by Vega et al. (2015). There were no carcinogenic effects in the long-term mouse study despite high dosages. Although this mouse study may be of relevance to Weight of Evidence and human relevance considerations, it is probably not a pivotal study for defining the Acceptable Daily Intake (ADI) in man. Therefore, only the rat carcinogenicity study is reported here.

This rat study and other toxicological data with synthetic astaxanthin from DSM constitute the principal toxicological data source for astaxanthin as a whole and are relevant to the toxicological regulatory evaluation of astaxanthin, independent of the manufacturing or production source. This publication here presents the data from recent genotoxicity studies as well as the less recent rat carcinogenicity study with synthetic astaxanthin.

For astaxanthin from biological sources, supplementation in various foods has been approved through the GRAS regulatory process in the USA, including FDA Notification (Fuji Chemical Industry Co, Ltd, 2009). This GRAS evaluation was based both on animal data and a human study with a dosage of 6 mg/day (Spiller and Dewell, 2003).

This is however not the highest dosage investigated in man. Dosages of up to 40 mg/day (equal to 0.67 mg/kg bw/day for a 60 kg human) have been administered for up to 2 months in other reported human clinical studies with no reported adverse effects (Andersen et al., 2007; Kim et al., 2011; Kupcinskas et al., 2008).

Recently the data for astaxanthin has been re-evaluated by the EFSA FEEDAP Panel (EFSA, 2014a) by using Benchmark Dose (BMD) analysis rather than traditionally derived NOAEL (No Observed Adverse Effect Level) values.

Although this publication has been prepared by a subsequent generation of toxicologists not involved with the supervision of the carcinogenicity study, the interpretation of the rat study still strongly reflects the opinion of the authors stated in the original study report.

With respect to genotoxicity studies, the original data set had comprised three studies. An Ames test (Chételat, 1981) and an in vivo Micronucleus Test (Gallandre, 1980) were performed with formulated astaxanthin (in a gelatine/starch beadlet matrix containing 10% astaxanthin) and an in-vitro cytogenetic assay with human lymphocytes (HLA) using crystalline astaxanthin (Strobel, 1987). These studies were negative for genotoxicity, which included absence of clastogenic activity, as was measured by the evaluation of metaphase chromosomes of human peripheral blood lymphocytes. The studies with formulated astaxanthin were not considered to be sufficient by EFSA, because the highest dose level recommended in the OECD guidelines 471 and 474 were not reached. In the negative in vivo Micronucleus Test in the mouse, dosages of 500, 1000 and 2000 mg/kg by oral application of the beadlet containing 10% astaxanthin, represented only 50, 100 and 200 mg astaxanthin/kg body weight respectively. In the in vitro cytogenetic assay high concentrations had not been achieved for solubility reasons (highest concentration tested, 120 μg/mL). EFSA, in their recent evaluation (EFSA, 2014a), used the more recently performed Ames test (Bellion and Verspeek-Rip, 2012) and in vitro Micronucleus Test (Bellion and Verbaan, 2013) with crystalline astaxanthin as the main studies for genotoxicity evaluation, as higher concentrations of astaxanthin had been achieved. These two in vitro genotoxicity studies are reported here.

2. Material and methods

2.1. Chemicals

Astaxanthin (3,3′-dihydroxy-β,β′-carotene-4,4′-dione, CAS No. 472-61-7) was prepared by DSM Nutritional Products Ltd. (Kaiseraugst, Switzerland) in different formulations. For genotoxicity